Deferred mortality: cyclic thermal stress during pupation triggers irreversible carry-over costs in a key pollinator

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Abstract

Extreme weather events, such as heatwaves, pose a concerning threat to global biodiversity, particularly to terrestrial ectotherms like insects. While lethal effects of thermal stress are widely studied, very little is known about sublethal carry-over effects of heatwaves experienced during development on adult fitness. Addressing this fundamental knowledge gap is crucial for key pollinators such as bumblebees, which face alarming population declines. Therefore, we used a highly controlled *in vitro* approach to expose *Bombus terrestris* pupae to ecologically relevant cyclic thermal stress, and studied their emergence success, subsequent adult longevity, and morphological traits. We found that cyclic thermal stress caused significant acute pupal mortality (up to 30% reduction in emergence). Most notably, this developmental stress resulted in deferred mortality, significantly reducing adult longevity (HR = 1.81) in both workers and males. Furthermore, we identified antennae and wing deformations as a powerful hazard indicator (HR = 2.50) that strongly predicts premature adult mortality. Our findings reveal that cyclic thermal stress during pupation imposes irreversible developmental damage that undermines adult physiological resilience. We argue that life-stage specific carry-over effects should be considered more in the future to avoid underestimating the long-term impact of climate change on crucial insect pollinator populations.

Keywords: climate change, heatwave, metamorphosis, mortality, survival, lifespan, deformations, malformations, social insects, bees

1. Introduction

Extreme weather events, such as heatwaves, are increasing in frequency and intensity due to climate change, leading to distinct and widespread implications for biodiversity, including insects (Meehl & Tebaldi 2004; Garcia *et al.* 2014). While thermal stress is known to have lethal and sublethal effects across a wide range of insects (González-Tokman *et al.* 2020; Ma, Ma & Pincebourde 2021; Jørgensen *et al.* 2022), most studies focus on the acute effects in adults. Little is known about how thermal stress during an earlier life-stage impose sublethal, yet long-lasting, carry-over effects on adult fitness (Zhang, Rudolf & Ma 2015; Klockmann, Kleinschmidt & Fischer 2017; Sales, Vasudeva & Gage 2021; Padde *et al.* 2024). Gaining a better understanding of such sublethal carry-over effects is essential for accurately forecasting population declines in keystone insect taxa such as bumblebees (*Bombus* sp., Hymenoptera: Apidae) (White & Dillon 2023b).

Bumblebees, as a cold-adapted taxon, have experienced populations declines, which are partially attributed to climate change (Cameron *et al.* 2011; Kerr *et al.* 2015; Soroye, Newbold & Kerr 2020; Martinet *et al.* 2021; White & Dillon 2023a; Ghisbain *et al.* 2024). This is a concerning trend, given that they provide crucial pollination services in ecosystems and agriculture (Klein *et al.* 2007; Goulson, Lye & Darvill 2008). While ground-nesting species can mitigate extreme ambient temperatures to some extent, other species, such as *B. hypnorum* and *B. pascuorum* that nest typically above the ground (Kevan, Rasmont & Martinet 2024) or commercially used species in greenhouses such as *B. terrestris* (Potts *et al.* 2016), face a higher risk of thermal stress. Colony-level studies have demonstrated that constant prolonged elevated temperatures negatively impact colony energetics, growth and reproductive fitness (Theodorou *et al.* 2022; Bretzlaff, Kerr & Darveau 2023; Bretzlaff, Kerr & Darveau 2024; Sepúlveda *et al.* 2024), and such thermal stress have been linked to altered offspring morphology (Gérard *et al.* 2018; Guiraud *et al.* 2021; Perl *et al.* 2022; Gérard *et al.* 2023). However, behavioural responses vary vastly between colonies and make it difficult to disentangle indirect effects (e.g.

fanning workers spend less time for nursing or foraging) from the direct physiological effects. To gain a precise mechanistic understanding of how thermal stress affects specific larval stages, *in vitro* rearing offers the required control at the cost of losing natural realism (Laußer & Kurze 2025; Wögler & Kurze 2025).

Our previous *in vitro* studies revealed stage-specific responses to thermal stress in *B. terrestris* (Laußer & Kurze 2025; Wögler & Kurze 2025). While smaller fourth-instar larvae may have a selective advantage under thermal stress due to their ability of more easily reaching critical body mass for pupation (Wögler & Kurze 2025), thermal stress at the pupal stage appears to select for individuals with increased relative lipid reserves (Laußer & Kurze 2025). Importantly, we found strong evidence for a reduction in adult emergence and ab increase of wing deformations in thermally stressed larvae and pupae (Laußer & Kurze 2025; Wögler & Kurze 2025). While these studies illustrate impaired larval and pupal development, the magnitude of the downstream impact on adult survival remains unknown.

To address this fundamental knowledge gap, we used highly controlled *in vitro* rearing of *B. terrestris* pupae to isolate the effect of cyclic thermal stress scenarios (simulating heatwaves) on individual life history. Our main aim was to assess the carry-over risk under fluctuating thermal extremes, an approach more ecologically relevant than constant temperature regimes (Radmacher & Strohm 2011). We focused on the pupal stage as metamorphosis in holometabolous insects, such as bees, represents the most extreme transition between phenotypes (Rolff, Johnston & Reynolds 2019) and is likely the most vulnerable to environmental stress (Laußer & Kurze 2025). We quantified both the direct effect on emergence success and the subsequent long-term adult survival, morphology, and energy reserves. We hypothesised that increasing cyclic thermal stress during pupation would affect adult morphology (specifically wing morphology (Laußer & Kurze 2025)) and trigger deferred mortality, significantly reducing adult longevity. Furthermore, we expected that the impact of thermal stress would differ between workers and males.

2. Materials and methods

2.1 Colony husbandry

We maintained ten queen-right source colonies of *B. terrestris* (Natupol Research Hives, Koppert B.V., Netherlands) under standardized laboratory conditions (Gilgenreiner & Kurze 2024; Laußer & Kurze 2025; Wögler & Kurze 2025). Colonies were housed in standard nest boxes (23 x 25 x 15 cm), which connected to foraging arenas (59 x 39 x 26 cm). We maintained all colonies at 25 ± 1°C and 30-50% RH, under a 14:10 h light:dark regime. Bumblebees had *ad libitum* access to a 66% w/w sucrose solution (Natupol Bee Happy, Koppert B.V., Netherlands). We supplied each colony with 6-11 g of pollen candy (67% w/w organic pollen, 25% w/w sucrose, 8% w/w water) daily.

2.2 Larvae collection and *in vitro* rearing

We collected 691 fourth-instar larvae (L4) from fully developed source colonies (figure S1*a*). Collecting L4 was crucial to ensure that we could start all thermal regimes consistently at the beginning of the pupal phase. Larvae were reared *in vitro* following our established protocol (for details see Laußer & Kurze 2025; Wögler & Kurze 2025).

Briefly, L4 were carefully transferred into 3D-printed polylactide (PLA) artificial brood cells (figure S1*b*), placed inside 24- or 48-well clear flat bottom plates (figure S1*c*; Falcon/Corning, USA), and put within a humidity-controlled plastic container (18.5 x 18.5 x 11.5 cm), kept at a constant temperature of 34°C (KB115, BINDER GmbH, Germany). To maintain a relative humidity (RH) of 65 ± 10%, a 120 mL cup of saturated sodium chloride solution was also placed within the container.

Larvae were fed a pollen medium (figure S1*b*), consisting of 50% w/v sucrose solution, 40% organic pollen, 10% yeast extract (Bacto™, BD, USA), and 1% casein sodium salt from bovine milk (Sigma-Aldrich, Germany) (Kato *et al.* 2022), twice daily to satiation (Wögler & Kurze 2025). Our setup allowed us to measure larval and pupal weight non-invasively and facilitated the easy removal of any dead individuals. Feeding was stopped with the beginning of the pupal phase.

2.3 Experimental procedure

At the beginning of pupation, each individual was weighed (d = 0.1 mg, Sartorius M-Pact AX224, Sartorius GmbH, Germany) and randomly assigned to one of three experimental thermal treatment groups (Table 1). Pupae were transferred to new, humidity-controlled containers within one of three programmed incubators. The control group was maintained at a constant 34°C (34°C ctrl). The two cyclic treatment groups experienced a 12:12 h temperature cycle between the base of 34°C (night phase) and a thermal maximum (day phase) of either 37°C (37°C cyclic) or 38°C (38°C cyclic). We monitored pupal survival and emergence success twice daily. In addition to the *in vitro* treatments, newly emerged adults (< 1 day old) were collected directly from their natal colonies to serve as an external colony control group (colony ctrl) for the subsequent longevity assay.

2.4 Adult survival assay

Newly emerged bees from the *in vitro* treatments and the additional colony control group (Table 1) were weighed and then individually housed in modified 50 mL tubes (figure S1*d*; Greiner Bio-one GmbH, Germany). These were stored in a wooden rack within the insectary room of their natal colonies at 25 ± 1°C and 30-50% RH (figure S1*e*). Each tube contained a 3D-printed PLA mesh insert for sanitary reasons. The lids were modified to include three 3 mm ventilation holes and one 1 cm feeding port, through which a 1.5 mL perforated tube provided *ad libitum* access to a 60% w/v sucrose solution. Pollen candy was offered separately in the caps of 2 mL reaction tubes and was replaced weekly or as necessary. Housing tubes and meshes were replaced as needed to ensure sanitary conditions. Adult survival was monitored around noon daily. Dead individuals were recorded and immediately frozen at -21°C until further analysis.

Table 1. Overview of the experimental design. This table details the rearing conditions (regime and temperature) of pupae for all experimental groups and an additional colony control. Sample sizes (n) reflect the total number of pupae per treatment at start of *in vitro* rearing experiment (indicated by [§]) and emerged adults at the start of the survival assay (indicated by [#]).

Treatment	Regime[§]	Temperature[§]	Sample size (n)
colony ctrl	colony reared	25 ± 1°C*	[#]115
34°C ctrl	constant	34°C	[§]132/ [#]102
37°C cyclic	12:12 h cycle	37°C:34°C	[§]125/ [#]82
38°C cyclic	12:12 h cycle	38°C:34°C	[§]146/ [#]78

* colonies kept in the insectary
[§] pupae / [#] adults

2.5 Morphological and physiological analysis

We contacted the morphological and physiological analysis following established protocols (Gilgenreiner & Kurze 2024; Laußer & Kurze 2025; Wögler & Kurze 2025). The intertegular distance (ITD), used as proxy for body size, was measured using a digital microscope (VHX-500F, Keyence GmbH, Germany). The sex of each bee was determined by counting flagellomeres (workers = 10; males = 11). Furthermore, any deformations of their antennae were recorded (figure S2*a*).

To assess wing integrity, forewings were carefully detached, embedded side-by-side on a microscope slide (Roti®Mount, Carl Roth GmbH + Co. KG, Germany) and photographed at 20x magnification. Wings were subsequently classified as deformed (figure S2*b*) when visually apparent malformations were present.

The remaining bee bodies were processed to determine dry mass and relative lipid content. Specimens were first drying at 60°C for three days (U40, Memmert GmbH & Co. KG, Germany) and immediately weighed to obtain their dry mass.

The absolute body lipid content was determined by using a petroleum ether extraction method. The specimens were re-drying and weighted again post-extraction to allowing us to calculate the difference between the initial and post-extraction dry masses. The relative lipid content, which served as a proxy for the fat body size and energy reserves, was calculated as the absolute lipid content divided by the initial dry mass.

2.6 Statistical analyses

All statistical analyses and data visualizations were performed in R version 4.5.1 (R Core Team 2024), similar to our previous work (Kurze, Jenkins & Hughes 2020; Laußer & Kurze 2025). The complete code, including model selection procedures (R Markdown output), and datasets are provided in the Dryad Digital Repository (XXX).

To analyse the effect of cyclic thermal stress and additional explanatory variables (e.g. sex, pupal weight) and their interactions on emergence rates, ITD, dry mass, relative lipid content, as well as antennae and wing deformation, we fitted generalized linear mixed effect models (GLMMs) using the *glmmTMB* package (Brooks *et al.* 2017). Emergence rates and deformation status were fitted with binomial distribution (logit link), while the continuous variables ITD, dry mass, and relative lipid content were fitted with a gamma distribution (log link). For analysing the effects on pupation duration (days to emergence), we applied an ordinal cumulative link mixed model (CLMM) with the Laplace approximation using the *ordinal* package (Christensen 2019), as assumption for fitting a GLMM were not met (described below). While the CLMM included only colony ID as a random factor, all GLMMs also included collection date as random factor to account for colony and temporal-specific variability.

For each response variable, a set of candidate models was compared. The most parsimonious and best-fitting model based on the Akaike information criterion (AIC), with lower AIC values indicating a better fit. The best-fitting models were compared to a simpler nested model using likelihood ratio tests (LRTs). When added terms did not improve model fit significantly, the simpler model was chosen and compared against its respective null-model using LRT. Model assumptions and dispersion of the data were checked using the *DHARMa* package (Hartig 2020). When model assumptions were met, statistical significance ($p < 0.05$) was determined using the *Anova* function. Pairwise comparisons between treatment groups were conducted using the *emmeans* package (Lenth & Lenth 2018) with Holm-Bonferroni corrections. Smooth predictions of emergence

rate across the observed range of larval weights were generated using a prediction dataset with 100 evenly spaced values.

Differences in survival probability between treatment groups were initially determined for workers and males separately based on Kaplan-Meier analyses using the *survival* and *ggsurvfit* packages (Therneau & Grambsch 2000). To further analyse the main factors affecting adult survival, we fitted a set of candidate Cox Proportional Hazards (CoxPH) models the using *coxme* packges (Therneau & Grambsch 2000). The full model contained treatment and sex (and their interactions), adult dry mass, relative lipid content, and deformations (antennae and wing deformation combined). The colony ID was included as a random factor. Model selection followed the AIC and LRT procedure as described above. The final best-fitting model to predict mortality included the explanatory variables treatment, sex, and their interactions, alongside deformations status. As proportional hazards (PH) assumptions were not entirely met (as diagnosed model diagnostics using *ggcoxdiagnostics* function of the *surmiver* package (Kassambara, Kosinski & Biecek 2016)), the final model was stratified by sex. Pairwise comparisons between treatment groups were performed using the *glht* function with Holm-Bonferroni correction.

3. Results

3.1 Emergence success and developmental duration

Cyclic thermal stress and pupal weight significantly affected the proportion of pupae reaching adulthood (GLMM: χ^2 = 35.187, df = 3, p < 0.0001; figures 1*a,b*). This effect was primarily driven by the increase in thermal stress (χ^2 = 22.276, df = 2, p < 0.0001; figure 1*a*). Pairwise comparisons revealed that pupae exposed to the 38°C cyclic treatment resulted in a 30% lower likelihood of emergence compared to the controls (p < 0.0001), while the 37°C cyclic treatment had a 15% reduction in emerged adults (p < 0.05). The difference between the 38°C and 37°C cyclic treatments was also significant (p < 0.05). Furthermore, emergence probability was significantly higher with an increase in initial pupal weight (χ^2 = 11.065, df = 1, p < 0.001; figure 1*b*).

While we observed a significant overall effect on pupation duration (CLMM: χ^2 = 43.958, df = 3, p < 0.0001; figure 1*c*), this effect was not related to the thermal stress treatment (χ^2 = 1.6121, df = 2, p > 0.05), but was sex-dependent (χ^2 = 34.311, df = 1, p < 0.0001). Pairwise comparisons revealed that workers of all treatment groups were significantly different from males of all treatment groups (p < 0.001).

3.2 Carry-over effects on adult longevity

Adult survival was significantly reduced with increasing cyclic thermal stress exposure during pupation for both workers and males (Log-rank test for workers: χ^2 = 8.9, df = 2, p = 0.01; for males: χ^2 = 11.1, df = 2, p < 0.01; Kaplan-Meier survival curves in figure 2*a,b*). The presence of carry-over effects on longevity was strongly supported by the CoxPH model stratified by sex (χ^2 = 349.78, df = 3, p < 0.0001, model concordance = 0.64; figure 2*e*). Adults of 38°C cyclic treatment had a significantly higher mortality risk than the controls (HR = 1.81, 95% CI = 1.56-2.13, p < 0.0001). The 37°C cyclic treatment posed a marginally significant increase in mortality risk (HR = 1.30, 95% CI = 0.98-1.73, p = 0.07). We also found that individuals with morphological deformations (combining

deformed antennae and wings) had a significantly higher mortality risk (HR = 2.50, 95% CI = 2.02-3.10, $p < 0.0001$).

Importantly, we found no significant difference in adult survival between *in vitro* reared controls and naturally colony reared controls (workers: $\chi^2 = 0$, df = 1, $p > 0.05$, figure 2*c*; males: $\chi^2 = 0.8$, df = 1, $p > 0.05$, figure 2*d*).

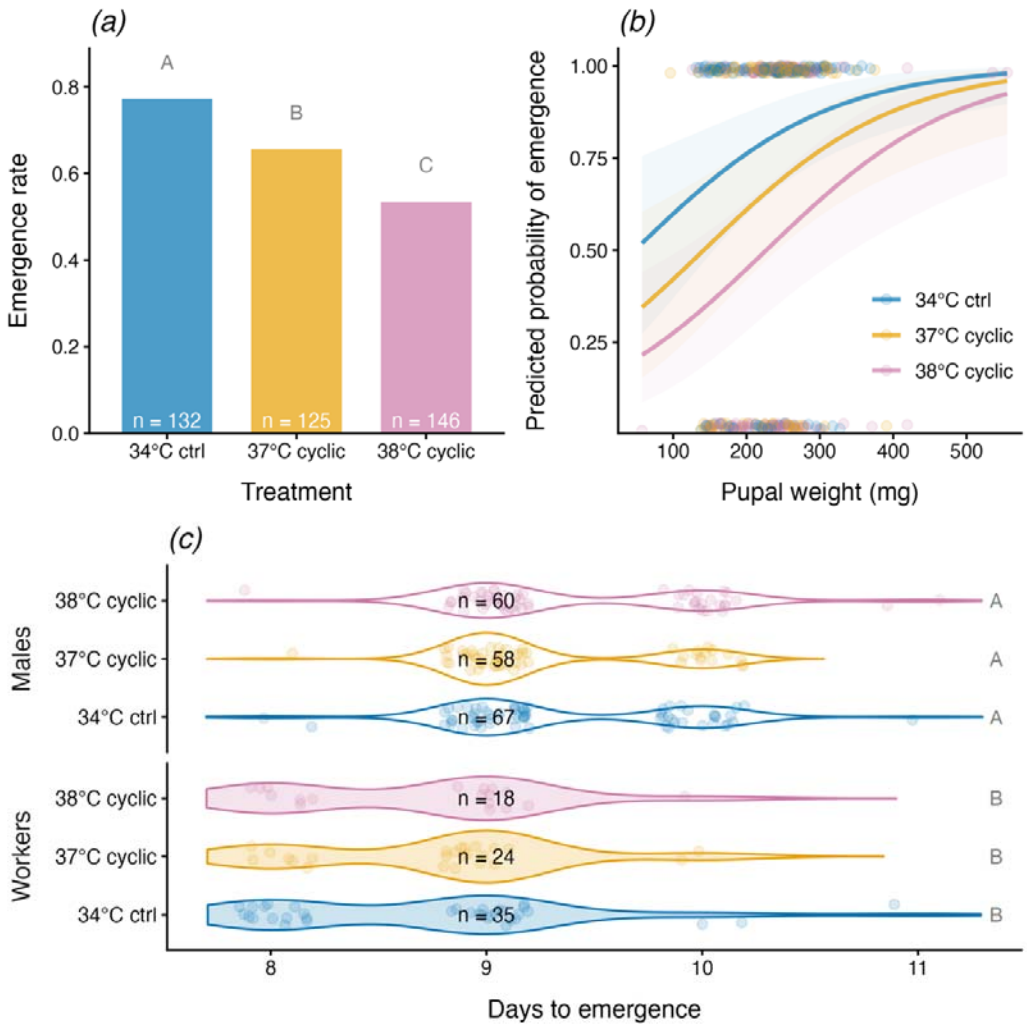

Figure 1. Direct effects of cyclic thermal stress during pupation on emergence success and development duration in *B. terrestris*. Panel *(a)* illustrates the proportion of pupae emerging as adult bees, and *(b)* shows the predicted probability of emergence in relation to pupal weight and treatment. Panel *(c)* displays the pupation duration until emergence for male (open violins) and workers (filled violins). Controls (34°C ctrl, reared at constant 34°C, blue) were compared to individuals exposed to cyclic thermal stress regimes of 37°C (37°C cyclic, orange) and 38°C (38°C cyclic, reddish-purple). Individual data points in (*b*) and (*c*) are shown as circles. In panels (*a*) and (*b*), sample sizes (n) are provided in plots. Significant differences ($p < 0.05$), based on post-hoc comparisons from GLMMs, are indicated by different letters above the bars and next to the violin plots (groups sharing a letter are not significantly different).

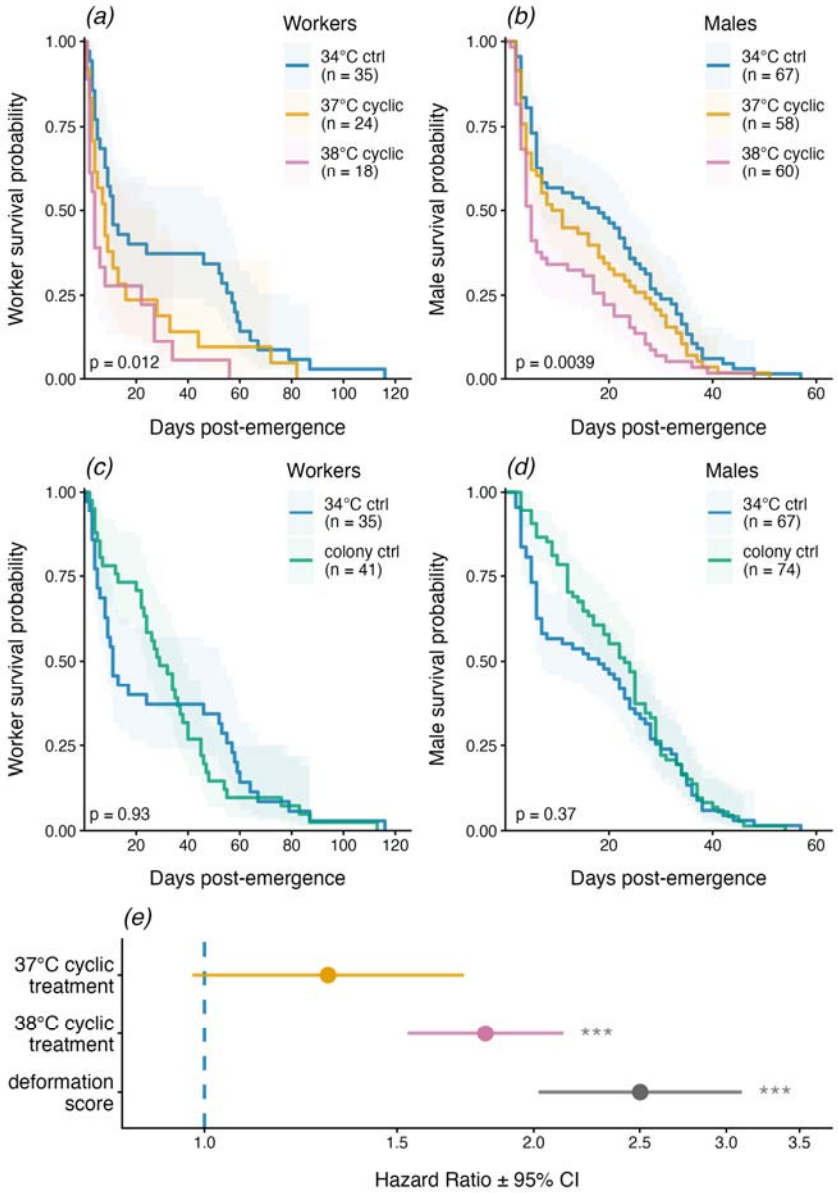

Figure 2. Carry-over effects of cyclic thermal stress during pupation on adult longevity in *B. terrestris*. Kaplan-Meier survival curves (± 95% confidence intervals, CI, shaded areas) showing the effect of in vitro thermal treatments on worker (*a*) and male (*b*) adult longevity. The constant 34°C control group (34°C ctrl, blue) is compared to the cyclic thermal stress treatments (37°C cyclic, orange; 38°C cyclic, reddish-purple). Panel (*c*) and (*d*) compare the survival of *in vitro*-reared controls (34°C ctrl) to naturally reared colony controls (colony ctrl, bluish green) for worker and male, respectively. Sample sizes (n) and overall statistical significance (p) from the Kaplan-Meier analyses are provided on each plot. Panel (*e*) shows a forest plot of the hazard ratios (HR) ± 95% CI based on the stratified CoxPH model for treatment and morphological deformations (combined antennae and wings deformations; figure S2) on longevity. HR values > 1 indicate significantly increased hazard. Asterisks denote significance (*** $p < 0.001$) relative to the 34°C ctrl baseline group (dashed blue line).

3.3 Morphology

We found evidence that thermal stress treatment during pupal development increased the likelihood of adults having deformed wings (GLMM: $\chi^2 = 12.791$, df = 2, $p < 0.01$; figures 3*a,* filled bars). Pupae of the 38°C cyclic treatment developed 3.6-fold more deformed wings compared to the controls ($p < 0.01$). This difference was only marginally significant compared to individuals of the 37°C cyclic treatment ($p = 0.08$). Individuals of the 37°C cyclic treatment developed non-significantly more deformed wings compared to the controls ($p > 0.05$). We found also no evidence that thermal stress developed significantly more deformed antennae ($\chi^2 = 2.298$, df = 2, $p > 0.05$; figures 3*a,* open bars).

While we found a significant effect on adult body size metrics ITD ($\chi^2 = 32.155$, df = 3, $p < 0.0001$; figure 3*b*) and dry mass ($\chi^2 = 32.155$, df = 3, $p < 0.0001$; figure 3*c*), this effect was primarily sex-dependent (ITD: $\chi^2 = 37.343$, df = 1, $p < 0.0001$; dry mass: $\chi^2 = 32.155$, df = 3, $p < 0.0001$). Although treatment had no effect on the ITD ($\chi^2 = 1.097$, df = 2, $p > 0.05$), there was a weak interaction effect between treatment and sex on adult dry mass ($\chi^2 = 8.827$, df = 2, $p < 0.05$). Pairwise comparisons revealed strongest effects between workers of the 34°C control group compared to males of all treatment groups (34°C ctrl: $p < 0.0001$; 37°C cyclic: $p < 0.0001$; 38°C cyclic: $p < 0.001$). Males of the control group were also significantly different from workers in the cyclic treatments (37°C cyclic: $p < 0.01$; 38°C cyclic: $p < 0.01$).

3.4 Energy reserves

For the analysis of the effects on the relative lipid content at the end of their adult life, our model including treatment and sex was not significant ($\chi^2 = 5.16$, df = 3, $p > 0.05$, figure 3*d*). Within this model, we found a small but significant effect of sex on the relative lipid content ($\chi^2 = 4.412$, df = 1, $p < 0.05$), whereas treatment had no effect ($\chi^2 = 0.921$, df = 2, $p > 0.05$).

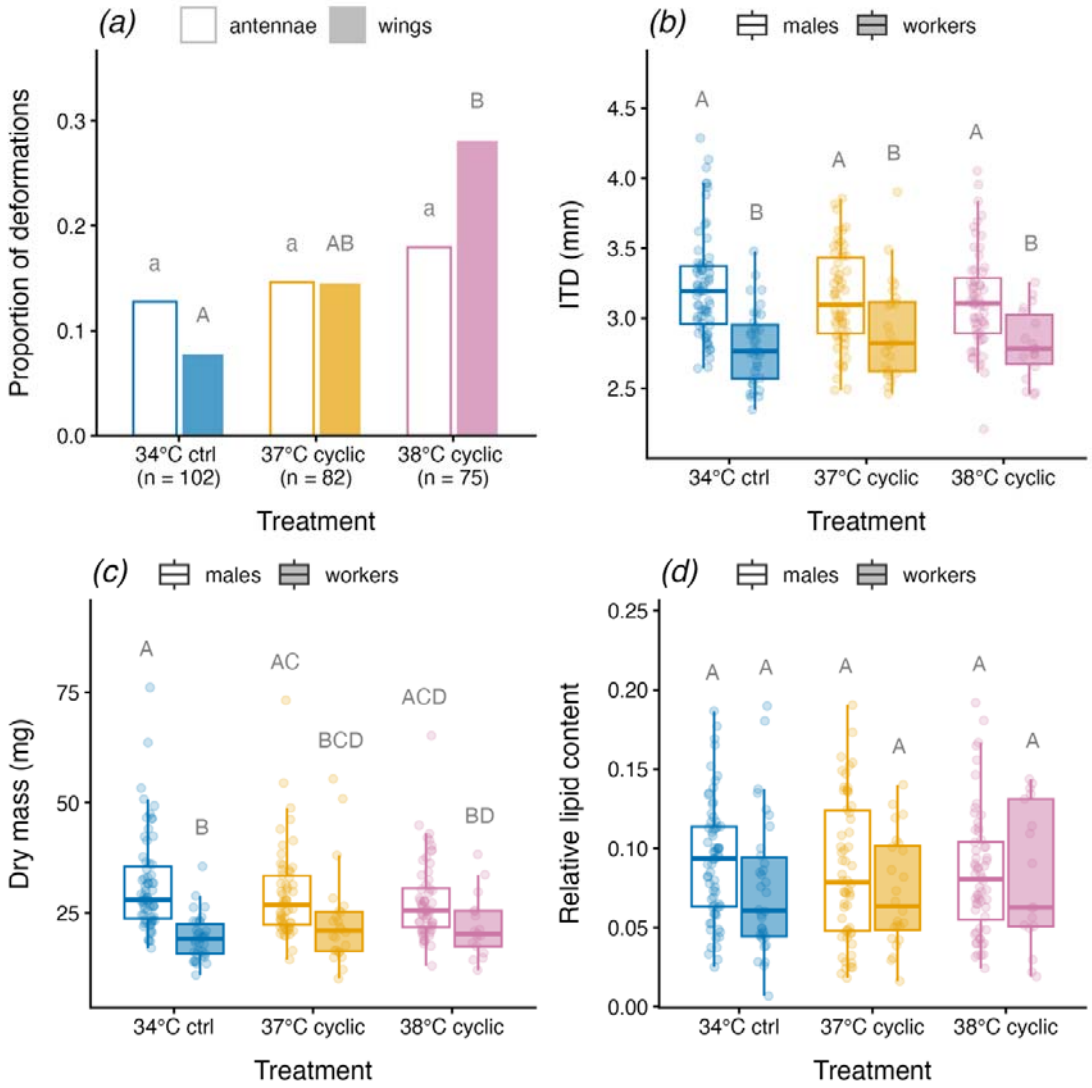

Figure 3. Effects of cyclic thermal stress during pupation on morphological and physiological traits in adult *B. terrestris*. Panel *(a)* illustrates the proportion of individuals with deformed antennae (open bars; figure S2*a*) and wings (filled bars; figure S2*b*), both separately analysed. Sample sizes (n) are provided below for each treatment group. Box plots (*b*-*d*) show the treatment effects (constant 34°C ctrl, blue; 37°C cyclic, orange; 38°C cyclic, reddish-purple) on body size, quantified by intertegular distance (ITD) and dry mass, and relative lipid content as metric for energy reserves, in males (open boxes) and workers (filled boxes). Individual data points are displayed as circles. Significant differences ($p < 0.05$), based on post-hoc comparisons from GLMMs, are indicated by different letters above the bars and boxes (groups sharing a letter are not significantly different).

4. Discussion

Our study addresses a fundamental knowledge gap concerning the sublethal, long-term carry-over effects of thermal extremes in insect pollinators (González-Tokman *et al.* 2020; Ma, Ma & Pincebourde 2021; White & Dillon 2023b). To our knowledge, we provide the first evidence that thermal stress during pupal development leads to deferred mortality at the adult life-stage in bumblebees. This finding reveals a novel and previously unrecognized mechanism that may contribute significantly to pollinator decline. Specifically, cyclic thermal stress not only caused acute pupal mortality (i.e. reduced emergence; figure 1*a*) but also resulted in significantly reduced adult longevity (HR = 1.81) in both workers and males of *B. terrestris* (figures 2*a,b,e*). This finding suggests that thermal stress during pupation imposes irreversible developmental damage that undermines adult physiological resilience.

Developmental bottlenecks and irreversible damage

The high sensitivity observed during the pupal phase underscores its role as a critical developmental bottleneck. The reductions in emergence success from cyclic thermal stress (15% at 37°C and 30% at 38°C cyclic stress; figure 1*a*) confirm patterns previously observed under constant stress (Laußer & Kurze 2025). Our observation that heavier pupae were more likely to emerge (figure 1*b*) supports the idea that the pupae-to-adult transition might be resource-dependent (Laußer & Kurze 2025). Critically, this suggests that resource limitations within a colony could unleash negative synergistic interactions, amplifying the detrimental effects of heatwaves.

Most notably, 38°C cyclic thermal stress led to a higher incidence of deformed wings (figure 3*a*). While wing morphology is a known indicator of irreparable developmental damage (Vanderplanck *et al.* 2021; Laußer & Kurze 2025), here we demonstrate that antennae and wing deformations together can serve as a powerful hazard indicator (HR = 2.50) that predicts adult longevity (figures 2*e*). Beyond individual mortality, workers and males with severely deformed wings are unable to fly, which

consequently would reduce colony fitness. In fact, it has already been shown that thermal stress during development can reduce adult fitness across diverse insect taxa (Zhang, Rudolf & Ma 2015; Sales, Vasudeva & Gage 2021; Padde *et al.* 2024). Adding to the consequences of deformed wings, deformed antennae likely impair their ability to detect scents of nest mates, mating partners (important for males), and floral resources (important for workers). This idea would be supported by a study demonstrating heat stress-induced olfactory impairment in adult bumblebee workers (Nooten *et al.* 2024). Importantly, the observed survival curves showed no significant difference between *in vitro* reared controls and colony-reared controls, validating the application of our controlled *in vitro* rearing approach to natural contexts.

Decoupling fitness costs from adult body size and fat body

Our study provides evidence that deferred mortality was not associated with easily measurable morphological traits. Despite the direct impact on emergence, we observed no treatment-dependent effects on body size proxies (ITD or dry mass; figures 3*b,c*), in contrast to some colony-level studies (Gérard *et al.* 2018; Guiraud *et al.* 2021; Perl *et al.* 2022; Gérard *et al.* 2023). This result suggests that selection pressure from short-term thermal stress for body size might be more pronounced at earlier larval stages (Wögler & Kurze 2025) or is insufficient (Laußer & Kurze 2025). Alternatively, it would be plausible that body size effects observed in stressed colonies are an indirect result of altered worker nursing and foraging behaviour (Bretzlaff, Kerr & Darveau 2024; Sepúlveda *et al.* 2024), rather than a direct physiological consequence of the heat itself. This highlights the importance of our *in vitro* approach isolating the direct physiological effects from comprehensive colony dynamics (Laußer & Kurze 2025; Wögler & Kurze 2025).

While increased lipid content is advantageous for thermally stressed pupae to successfully emerge (Laußer & Kurze 2025), we found no significant difference in relative lipid content at the end of their adult life (figure 3d). This indicates the capacity of surviving adults to maintain homeostasis of their fat body, which is the primary site for heat shock protein (HSP) synthesis and storage of energy reserves (Arrese & Soulages 2010; King &

MacRae 2015; Vanderplanck *et al.* 2021), by resource allocation or compensatory foraging. Hence, any sublethal damage carrying over to adulthood might be genetically, structurally, and/or physiologically mediated rather than simply due depleted energy reserves. This aligns with findings where developmental thermal stress indirectly affected insect adult physiology, such as flight performance and behaviour (Arambourou, Sanmartín-Villar & Stoks 2017; Galarza *et al.* 2019; Gérard *et al.* 2022; Perl *et al.* 2022).

Conclusion

These findings highlight that sublethal thermal stress during insect development may lead to profound fitness costs. Our study provides compelling empirical evidence that cyclic thermal stress during pupation significantly reduces adult longevity. Furthermore, our findings confirm that 38°C is a critical threshold for development disruption in *B. terrestris* (Laußer & Kurze 2025; Wögler & Kurze 2025). Although our study simplifies realistic thermal stress scenarios, heatwaves with peak temperatures exceeding 38°C for several days are becoming more common in Europe (Barriopedro *et al.* 2011; Rita *et al.* 2020; Lhotka & Kyselý 2024). Bumblebee species which are less heat-tolerant than *B. terrestris*, such as *B. lapidarius* and *B. alpinus* (Martinet *et al.* 2021), and colonies nesting above ground would be expected to be especially at risk. As sublethal carry-over effects are rarely considered, current prediction models may underestimate the long-term impact of heatwaves on crucial pollinator populations. To generate a more holistic picture of environmental stress impact, gaining a nuanced mechanistic understanding of carry-over effects across diverse insect and life stages, incorporating both individual-level, and for social insects, also colony-level physiological and life-history data, remains crucial.

Statement of authorship:

M.M.M.: methodology, investigation, data curation. C.K.: conceptualization, methodology, investigation, data curation, resources, validation, formal analysis, writing – original draft preparation, writing – review and editing, visualization, supervision, project administration.

Data accessibility statement:

Dataset and R script used for statistical analysis and producing all figures are available from the Dryad Digital Repository: XXX. Electronic supplementary material is available online.

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Competing interests

The authors have no competing interests.

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