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Ontogeny shapes vulnerability to climate change underground: Larvae of a subterranean beetle are more sensitive to temperature increase than adults

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Abstract

Although the effects of climate change on biodiversity are increasingly well documented, subterranean ecosystems remain largely understudied. These environments, characterized by stable temperatures and permanent darkness, offer a unique opportunity to assess the vulnerability of low-dispersal species to warming. While recent studies have shown that subterranean invertebrates are highly sensitive to rising temperatures, most research has focused exclusively on adult stages, overlooking the thermal sensitivity of other life stages. Here, we compared the upper thermal limits (LT_{50}) of larvae and adults of *Anillochlamys tropica*, a cave-dwelling beetle endemic to the southeastern Iberian Peninsula. Individuals were exposed to constant temperatures ranging from 17 °C to 27.5 °C for seven days in controlled laboratory experiments. Adults tolerated temperatures up to 25 °C but died within 24 h at 27.5 °C. In contrast, larvae were significantly more sensitive, showing reduced survival at 23 °C and 25 °C, and exhibiting a lower LT_{50} (21.47 ± 0.53 °C) than adults (26.1 ± 0.73 °C). Notably, the current cave temperatures approach the larvae's thermal limit, meaning that the species could have a narrow thermal safety margin. These findings demonstrate that the early life stages of subterranean beetles are particularly vulnerable to climate warming and underscore the importance of considering all developmental stages when evaluating the impacts of climate change on subterranean biodiversity.

Keywords
Anillochlamys tropica, global warming, Leiodidae, subterranean ecosystems, thermal tolerance, upper thermal limit

Introduction

Global warming represents a major threat to biodiversity and ecosystem functioning (Grimm et al. 2013; Pecl et al. 2017; Harvey et al. 2023). However, subterranean ecosystems—which extend beneath at least 19% of Earth's land surface (Chen et al. 2017)—have been largely overlooked in conservation and climate change agendas (Sánchez-Fernández et al. 2021; Wynne et al. 2021). In recent years, the growing recognition of their ecological relevance and vulnerability has led to increasing scientific attention being paid to these ecosystems (Vaccarelli et al. 2023).

Subterranean environments are characterized by stable temperatures across daily, seasonal, and annual cycles, mirroring the mean annual surface temperature (Sánchez-Fernández et al. 2018). Their simplified ecological interactions and relative isolation make them ideal natural laboratories for investigating species sensitivity to climate change (Mammola et al. 2019b). While these habitats have driven convergent morphological adaptations in resident fauna (Howarth and Moldovan 2018), physiological traits have received comparatively less attention and have only recently begun to be explored, partly due to the logistical challenges of accessing and studying subterranean species (Mammola et al. 2019a).

Recent work on subterranean beetles of the tribe Leptodirini (family Leiodidae), which exhibit strongly reduced or simplified larval stages (Sendra and Baixeras 1984; Cieslak et al. 2014b), has shown that they generally possess low upper thermal limits (UTLs) and limited capacity for thermal acclimation compared to most surface-dwelling invertebrates (Rizzo et al. 2015; Mammola et al. 2019c; Pallarés et al. 2019, 2021). Moreover, heat tolerance tends to decrease with an increasing degree of subterranean specialization across subterranean lineages, with current habitat temperature apparently playing a minor role (Colado et al. 2022). Despite these advances, most studies to date have focused exclusively on adult stages, neglecting the earlier stages of the life cycle. In surface-dwelling invertebrates, it is well established that early developmental stages often exhibit greater sensitivity to environmental stressors, including temperature (Zhao et al. 2010; McCauley et al. 2018; Agyekum et al. 2021; Marochi et al. 2024). This highlights a critical gap in our understanding of how ontogenetic variation influences the thermal vulnerability of subterranean species.

To address this knowledge gap, we investigated the upper thermal limits of both larvae and adults of *Anillochlamys tropica* (Abeille de Perrin, 1881), a cave-dwelling beetle endemic to the southeastern Iberian Peninsula (Salgado and Fresneda 2003). This study aimed to provide, for the first time, empirical evidence of ontogenetic differences in thermal tolerance in a subterranean invertebrate. By identifying the most thermally sensitive life stages, our findings contribute to a more accurate assessment of species vulnerability to climate change in subterranean ecosystems.

Methods

Studied species, field collection and laboratory breading

This study focused on the cave beetle *Anillochlamys tropica* (Leptodirini, Leiodidae), whose life cycle involves two larval stages before adulthood (i.e., an intermediary cycle; Sendra and Baixeras 1984; Cieslak et al. 2014a, 2014b; Moldovan 2018). Adults were collected from Meravelles cave (Alzira, Valencia) (lat: 39.124927, long: -0.424798) in February 2024 and transported to the laboratory under cool, humid, and dark conditions to minimize stress. All specimens were randomly divided into two groups: one for adult thermal tolerance tests (N = 48) and another for breeding (N = 15). Both groups were acclimated to 17 °C and approximately 90% relative humidity (simulating cave conditions) in an incubator (Memmert m360, Germany) prior to the experiments, in humid containers (adult thermal tolerance tests) and Petri dishes (breeding adults) with clay, moss and baker's yeast as food. Eggs from the breeding group were monitored every two days. The first-instar larvae (N = 5 placed in Petri dish) obtained were used for thermal tolerance tests.

Thermal tolerance experiments

Thermal tolerance was assessed by exposing adults and larvae to constant temperatures (17 °C, 20 °C, 23 °C, 25 °C, and 27.5 °C) for 7 days, with relative humidity above 90% and in darkness. These values represent natural and extreme conditions, according to previous studies with subterranean Pyrenean Leptodirini (Rizzo et al. 2015; Pallarés et al. 2020, 2021; Colado et al. 2022). Each treatment included 8 to 10 adults or 13 to 20 larvae, with both groups placed in incubators (Memmert m360, Germany; Panasonic MLR-352H, Japan; pHcbi MLR-352-PE, Japan; and Sanyo MLR-351H, Japan) and monitored daily for survival. Environmental conditions were continuously recorded (HOBO MX230, Onset Computer Corporation, Bourne, USA data loggers).

Data analysis

Survival of adults and larvae was analyzed using Kaplan–Meier curves (Altman, 1990; Therneau, 2015a) and Cox models (R package: coxme v2.2-20) (Therneau and Grambsch 2000, Therneau 2015b) to test the effects of temperature, life cycle stage, and their interaction. Given a significant interaction, post-hoc analyses were performed (R package: emmeans v1.10.3) (Nordstokke and Stelnicki 2024) to specifically determine at which temperature treatment survival differed between stages. The upper thermal limit (LT_{50}, temperature causing 50% mortality after 7 days) was estimated with a binomial GLM using R package: *brglm* version 0.7.2.

Data availability

Datasets for the analyses performed in this study are available for download on Figshare (https://doi.org/10.6084/m9.figshare.28706111).

Results

Temperature, life cycle stage, and their interaction significantly affected *A. tropica* survival (Table 1). Adults maintained high survival (90–100%) at 17–25 °C for 7 days, but all died within one day at 27.5 °C (See Suppl. material 1: table S1, Fig. 1A). Larvae showed 100% and 73% survival at 17 °C and 20 °C, respectively, while exposure to 23 °C and 25 °C caused progressive mortality (~80% by day 7). No larvae survived beyond one day at 27.5 °C (See Suppl. material 1: table S1, Fig. 1B). The mean probability of mortality did not differ between stages at 17 °C and 20 °C, but larvae showed significantly higher mortality than adults at 23 °C and 25 °C (See Suppl. material 1: table S2). Estimated LT_{50} values after 7 days were 26.1 ± 0.73 °C for adults and 21.47 ± 0.53 °C for larvae (See Suppl. material 1: table S1).

Table 1. Results of the Cox model to assess the effects of temperature and life cycle stage on the probability of mortality of *Anillochlamys tropica*. Significant effects (p-value ≤ 0.05) are marked in bold.

	χ^2	df	p-value
Life cycle stage	16.55	1	**<0.001**
Temperature	140.48	4	**<0.001**
Life cycle stage x Temperature	16.31	4	**0.003**

χ^2: chi-square statistic, df: degrees of freedom.

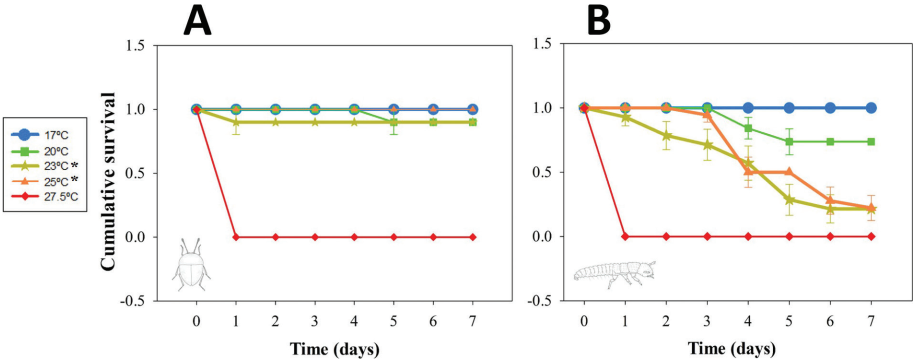

Figure 1. Kaplan-Meir survival curves for the five temperature treatments used to measure the upper lethal limits of adult (**A**) and larvae (**B**) specimens of *Anillochlamys tropica*. Each point represents the probability of survival (mean ± SE) in each treatment over the 7 days of exposure. Significant differences in the probability of mortality between larvae and adults in each of the temperature treatments (p-value ≤ 0.05 in post-hoc analyses) are indicated with asterisks.

Discussion

Our results show that the thermal tolerance of *A. tropica* (in both larvae and adults) is notably lower than that of most surface insects (Bennett et al. 2018). Remarkably, the upper thermal limits are higher than those obtained for the other subterranean Leiodidae adult species studied so far (mean LT_{50} around 21 °C) (Pallarés et al. 2019; Colado et al. 2022). This relatively high thermal tolerance may be influenced by the comparatively warm cave conditions of this species (17 °C), suggesting that local habitat temperatures could play a greater role in shaping heat tolerance than previously assumed (Colado et al. 2022). To disentangle the relative contributions of habitat temperature, evolutionary history, and degree of subterranean adaptation in shaping thermal tolerance, broader comparative studies across multiple lineages and environmental contexts are needed.

In this study, we provide the first estimate of thermal tolerance for the larval stage of a subterranean species, overcoming the significant logistical challenges faced when collecting larvae in cave environments or rearing them under laboratory conditions. Our key finding was that larvae were significantly more sensitive to heat than adults, with an LT_{50} that was nearly 5 °C lower. This difference may be due to higher metabolic demands due to their smaller size and investment in growth, limiting their ability to activate energetically costly thermal regulation mechanisms, such as the production of heat stress proteins (Krebs and Loeschcke 1994). Not mutually exclusive, their thinner cuticle compared to adults may imply higher sensitivity to desiccation and heat stress (Delaurance 1963; Chown and Nicolson 2004). Such ontogenetic differences in thermal sensitivity are likely widespread among subterranean insects and highlight the need to assess all life stages when evaluating vulnerability to climate change (Bowler and Terblanche 2008; Kingsolver et al. 2011; Kingsolver and Buckley 2020).

The upper thermal limit of the larvae is only a few degrees above the current cave temperature, leaving a narrow thermal safety margin (i.e., the difference between the upper thermal limit and habitat temperature; see Clusella-Trullas et al. 2021), making them especially vulnerable to further warming. Unlike most surface-dwelling species, subterranean beetles cannot avoid heat through migration due to their limited dispersal capacity. In some surface species, thermal sensitivity in the early life stages can often be mitigated by synchronizing development with seasonal climate patterns, allowing larvae to avoid the warmest periods of the year (Powell and Logan 2005). However, in deep subterranean ecosystems, where seasonal temperature fluctuations are minimal, this strategy is not feasible, nor is behavioral thermoregulation by microhabitat selection, given the homogeneous and stable cave environment. Projected temperature increases in the Mediterranean region could further threaten their persistence (MedECC 2020; Todaro et al. 2022).

Although survival studies provide valuable information for assessing persistence under climate change, the predictive potential of these data is still limited (Colado et al. 2024). Although this study provides an important first step in understanding thermal sensitivity across life stages in subterranean beetles, it has several limitations. We focused solely on first-instar larvae, leaving the responses of other developmental stages, such as eggs, second instar, and pupae, unexplored. This was due to logistical constraints to have enough adults reproducing at the same time to obtain both eggs and larvae sufficient to reach stage II and even complete the life cycle. Moreover, our assays measured only lethal effects, whereas sublethal impacts such as reduced growth or delayed development may occur at lower, non-lethal temperatures.

Thermal sensitivity often occurs in a hierarchical manner, such that processes most sensitive to environmental change can limit the overall fitness of an organism, and survival is often possible over a wider range of temperatures than locomotion, development or reproduction (Buckley and Kingsolver 2012; Evans et al. 2015). It is also critical to consider exposure time, as previous studies have shown that the sub-lethal effects of thermal stress in cave beetles are time-dependent (Pallarés et al. 2020). We also did not account for interactions with other key environmental stressors, such as ambient humidity in isolation or in combination with temperature. This factor (which is highly constant and near saturation in subterranean habitats) could influence the survival and thermal tolerance of subterranean species. Notably, Sendra et al. (2025) reported a decline in *A. tropica* populations, which was suggested to be related to a decline in relative humidity. The cave they inhabit was likely no longer a suitable refuge due to the current dry conditions and degradation, with relative humidity records reaching up to 75%. To build on these findings, future research should investigate the sublethal effects on fitness-related traits, examine all developmental stages, incorporate multiple interacting environmental variables, and integrate laboratory data with detailed cave microclimate modeling to better predict species persistence under climate change.

Conclusions

Our findings revealed, for the first time, significant ontogenetic differences in thermal tolerance in cave-dwelling beetles, with larvae exhibiting significantly lower upper thermal limits than adults. This indicates that early developmental stages may be particularly susceptible to even moderate temperature increases, including cave environments currently considered relatively warm. As a result, the threat posed by climate change to Mediterranean subterranean biodiversity may be more severe and underestimated than previously recognized. To enhance predictions of species persistence and inform effective conservation strategies, we strongly advocate future research to (i) assess all developmental stages, (ii) evaluate responses to sublethal thermal stress, and (iii) incorporate fine-scale microclimatic variability within cave systems.

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Supplementary material 1

Supplementary information

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