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Loss of heat acclimation capacity could leave subterranean specialists highly sensitive to climate change

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Complete List of Authors:	Pallarés, Susana; University of Plymouth, Marine Biology and Ecology Research Centre; Universidad de Castilla-La Mancha - Campus de Toledo, Instituto de Ciencias Ambientales (ICAM) Colado, Raquel; Universidad de Murcia, Ecología e Hidrología; Universidad de Castilla-La Mancha - Campus de Toledo, Instituto de Ciencias Ambientales (ICAM) Botella-Cruz, Maria; Universidad de Murcia, Ecología e Hidrología Montes, Aitor; Basque Society for Biology Conservation; Cuevas de Oñati-Arrikrutz Balart-García, Pau; Instituto de Biologia Evolutiva Bilton, David; University of Plymouth, Marine Biology and Ecology Research Centre; University of Johannesburg, Zoology Millán, Andrés; Universidad de Murcia, Ecología e Hidrología Ribera, Ignacio; Instituto de Biologia Evolutiva Sánchez-Fernández, David; Universidad de Murcia, Ecología e Hidrología
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Loss of heat acclimation capacity could leave subterranean specialists highly sensitive to climate change

Short title: Acclimation capacity in subterranean specialists

Susana Pallarés[1,2], Raquel Colado[2,3], María Botella-Cruz[3], Aitor Montes[4,5], Pau Balart-García[6], David T. Bilton[1,7], Andrés Millán[3], Ignacio Ribera[6†], David Sánchez-Fernández[2,3]

[1]Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, University of Plymouth, Plymouth, UK

[2]Instituto de Ciencias Ambientales, Universidad de Castilla-La Mancha, Toledo, Spain

[3]Departamento de Ecología e Hidrología, Universidad de Murcia, Murcia, Spain

[4]Basque Society for Biology Conservation, Guipúzcoa, Spain

[5]Cuevas de Oñati-Arrikrutz, Guipúzcoa, Spain

[6]Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Barcelona, Spain

[7]Department of Zoology, University of Johannesburg, Johannesburg, South Africa

Corresponding author:

Susana Pallarés (susana.pallares@plymouth.ac.uk)

Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA, UK

Tel: +44 1752 584608

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† Deceased April 15, 2020

Abstract

Physiological traits are key in determining the vulnerability of narrow range, highly specialised animals to climate change. It is generally predicted that species from more stable environments possess lower thermal tolerance breadths and thermal plasticity than those from more variable habitats – the so-called 'climatic variability hypothesis'. However, evolutionary trade-offs between thermal breadth and its plasticity are also seen in some taxa, and the evolution of thermal physiology remains poorly understood. Subterranean environments are excellent systems for exploring these issues, being characterized by stable climatic conditions, with environmental variability increasing predictably from deep to shallow habitats. Acclimation capacity will be fundamental in determining the sensitivity of subterranean species to climate change, since they have poor dispersal capacity and limited possibility to exploit thermally different microhabitats in the uniform cave environment. We assessed critical thermal maximum (CT_{max}) and short-term heat acclimation capacity in three related beetles (Leiodidae: Leptodirini) with differing degrees of specialisation to the subterranean environment (deep, shallow and facultatively subterranean, respectively) and therefore exposed to contrasting thermal variability in nature. Only the facultative subterranean species showed any acclimatory capacity, also having the highest CT_{max} across the taxa studied. However, this species might experience the highest thermal stress in its habitat under climate change. The studied subterranean specialists will be poorly able to cope physiologically with temperature increase, but in contrast exposed to lower magnitude and rate of warming. Our results fit the climatic variability hypothesis, suggesting that adaptation to cave conditions has selected against the retention of acclimation mechanisms. We show that the pathways that determine vulnerability of subterranean species to climate change depend on their degree of specialisation to deep subterranean environments. This information, combined with evaluation of exposure to climatic changes at their present locations, is fundamental in identifying species or populations at greatest risk.

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Keywords: critical thermal maximum, physiological plasticity, thermal tolerance, climate variability hypothesis, subterranean environment, conservation, cave beetles, climate change

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Introduction

It is broadly recognised that physiological data may contribute directly to conservation in the face of climate change and advance the field of conservation physiology (Wikelski & Cooke, 2006; Cooke & O'Connor, 2010; Cooke *et al*., 2013; Evans, 2015). Indeed, accurate assessments of species vulnerability to climate change require integrated measurements of its different determinants, including exposure (the magnitude and change of climatic change) and factors of organisms' sensitivity at the individual level, such as the physiological tolerance to environmental change (Williams *et al*., 2008; Foden *et al*., 2019). In this context, the acclimation capacity of organisms determines their ability to track changing environmental conditions and is of fundamental importance, especially for species that are unable to respond to climate change by other means (e.g. range shifts, behavioural adjustments or evolutionary adaption) (Stillman, 2003; Seebacher, White, & Franklin, 2015). Thermal acclimation occurs when individuals adjust their physiology to compensate for the effects of temperature variation, and may ultimately extend critical thermal limits for performance and survival (e.g. Da Silva, Riginos & Wilson, 2019).

Despite multiple hypotheses proposed to understand how thermal plasticity varies across taxa (see Rohr *et al*. (2018) for a recent review), empirical studies suggest that acclimation responses are highly context-dependent. According to the climatic variability hypothesis (CVH) (Stevens, 1989), species from more stable environments are predicted to have lower thermal tolerance (i.e. narrower tolerance breadths and less acclimation capacity) than those from more variable habitats (e.g. Feder, 1978; Tomanek, 2008; Magozzi & Calosi, 2015; Shah, Funk, & Ghalambor, 2017; Markle & Kozak, 2018), but such a pattern has not always been supported (Seebacher *et al*., 2015). Furthermore, evolutionary trade-offs may constrain the evolution of the different components of thermal tolerance, so that species that have evolved the greatest thermal limits

(particularly upper ones) may have done so at the expense of the plasticity of such limits and therefore show limited acclimation capacity (e.g. Stillman, 2003).

Testing between such alternatives is challenging, given the multiple intrinsic and extrinsic factors that affect the range of temperature actually experienced by organisms. For the same reason, teasing apart the relative importance of the different drivers of vulnerability to climate change can be challenging. Climatically stable and isolated systems are excellent models to minimize confounding effects and focus on physiology in a climate change context. This is the case with subterranean habitats, where environmental conditions are more stable and homogeneous than at the surface, limiting the possibility of behavioral adjustments of their inhabitants through differential microhabitat use (Sánchez-Fernández *et al*., 2018). The dispersal potential is also extremely reduced for most subterranean specialists (Juan & Emerson, 2010; Rizzo *et al.*, 2017). Moreover, low genetic variability (Juan *et al*., 2010), low reproductive rates and long life cycles (Voituron *et al*., 2010) may constrain rapid evolutionary change in such taxa (Sánchez-Fernández *et al*., 2016; Mammola *et al*., 2019b). Therefore, as with species in many other isolated ecosystems (e.g. mountain summits or islands), they rely strongly on physiological adjustments to cope with environmental changes, even though the magnitude and rate of such changes in subterranean habitats is moderate compared to the surface (Domínguez-Villar *et al.*, 2015; Pipan *et al.*, 2018). Deep subterranean habitats typically experience very stable climates (but see Trontelj, Borko, & Delić, 2019) and within caves, such stability decreases markedly from inner to outer sections (Cigna, 2002; Badino, 2010; Pipan *et al*., 2010), presenting an ideal setting in which to explore the relationship between thermal tolerance and climatic variability and test macrophysiological hypotheses (Gaston *et al*., 2009; Sánchez-Fernández *et al*., 2018; Mammola *et al*., 2019b).

The rarity of many subterranean taxa (Sánchez-Fernández *et al*., 2018; Trontelj *et al*., 2019) and difficulties of access for sampling (Raschmanová *et al*., 2018; Castaño-Sánchez, Hose, &

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Reboleira, 2020; Mammola, *et al*., 2019a), have limited research on subterranean species to date. In terms of thermal tolerance, most of the data on subterranean organisms come from studies conducted on a handful of species and have generally shown they have a high thermal sensitivity: upper lethal limits are usually not higher than 23ºC (e.g. Issartel *et al*., 2005; Rizzo *et al*., 2015; Pallarés *et al.*, 2019) and studies based on molecular biomarkers indicate that thermal stress onsets at lower temperatures (Mermillod-Blondin *et al*., 2013; Pallarés *et al*., 2020). Several studies have also suggested that the degree of specialisation to the subterranean environment is negatively correlated with thermal tolerance breadth (Lencioni, Bernabò & Latella, 2010; Bernabò *et al.*, 2011; Novak *et al*., 2014; Raschmanová *et al*., 2018; Mammola *et al*., 2019c), broadly supporting the CVH. Whether physiological plasticity is reduced in the process of specialisation to subterranean life and how it could affect species sensitivity to climate change remains an open question, however, since most studies have ignored acclimation capacity (but see Rizzo *et al*., 2015; Pallarés *et al*., 2019, 2020). In addition, such studies have often involved disparate, unrelated species, sometimes from localities with very different climates, making meaningful comparisons difficult. Efforts to increase our knowledge on the thermal tolerance of subterranean fauna are needed, as they represent an important and typically endemic component of the global animal biodiversity, but still largely neglected in conservation programs.

In Leptodirini cave beetles (Coleoptera, Leiodidae), one of the most extreme modifications associated with underground colonisation is a reduction in the number of larval instars in the life cycle, likely as an adaptation to a resource-poor environment (Cieslak, Fresneda, & Ribera, 2014a, b). Species that can live in deep forest litter or the upper layers of fractured soil (MSS, *Milieu Souterrain Superficiel* (Juberthie, Delay, & Bouillon, 1980a,b, 1981; Mammola *et al*., 2016)), i.e., not necessarily confined in caves, typically have the standard life cycle of Coleoptera, with three larval instars (Minelli & Fusco, 2013). Deep or shallow subterranean

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specialists (true troglobionts *sensu* Sket (2008)) may show a reduction to two or -in the most extreme specialisation- one larval instar. Therefore, the number of larval instars in these species, clearly associated with the degree of subterranean specialisation, can be used as an indirect proxy for the degree of thermal stability species experienced in their evolutionary history. Here we assess critical thermal maximum (CT_{max}) and short-term heat acclimation capacity in three related Leptodirini taxa from areas with similar climatic histories but with life cycles of one, two and three larval instars respectively, reflecting different degrees of specialisation to the subterranean environment and hence exposed to contrasting thermal variability. We explore whether support for the CVH in subterranean taxa (Latella, Bernabò & Lencioni, 2008; Lencioni *et al*., 2010; Novak *et al*., 2014; Raschmanová *et al*., 2018; Mammola *et al*., 2019c) also holds for thermal plasticity. Under the CVH, both CT_{max} and acclimation capacity are expected to decrease with increasing subterranean specialisation. However, if trade-offs between thermal limits and plasticity have shaped the evolution of thermal tolerance in these taxa, such a relationship with subterranean specialisation might not be so evident for acclimation capacity (i.e., species living in the more variable habitats could tolerate higher temperatures but show little thermal plasticity). Extending these general hypotheses to the subterranean environment can provide important insights into the evolution of thermal physiology associated with the process of underground specialisation, as well as relevant information for the conservation of specialist animal taxa with limited dispersal capabilities.

Material and methods

Study species and collection

The study species belong to monophyletic lineages of the beetle tribe Leptodirini; the Pyrenean (*Speonomites crypticola* and *Bathysciola rugosa*) and Cantabrian clades (*Speonomidius crotchi*) (Salgado, Blas, & Fresneda, 2008; Ribera *et al*., 2010). Rearing experiments revealed that *S.*

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crotchi has a life cycle of two larval instars (Jeannel, 1911; Glaçon, 1955), whilst *S. crypticola* and *B. rugosa* were inferred to have one and three larval instars, respectively, based on robust ancestral state reconstructions (Cieslak *et al.*, 2014b). Two of the species are obligate cave inhabitants only found in deep subterranean habitats (*S. crypticola*) or in both deep and shallow subterranean environments (*S. crotchi*), whilst *B. rugosa* is a facultative subterranean species, most commonly found in deep forest litter, always in dark conditions (Salgado *et al*., 2008).

Live adults were collected in different sites within the same geographical region (Northern Spain), with broadly similar mean annual surface temperatures (Table 1), which are known to closely approximate the constant temperatures recorded inside caves (Moore & Sullivan, 1964; Smithson, 1991; Badino, 2004, 2010). Therefore, all species live under similar average climatic conditions but occupy habitats with different daily and seasonal climatic variability. The two subterranean species can be assumed to be exposed to an annual variation of a few degrees (≤ 4º C) around mean annual surface temperatures, which is the typical thermal range in caves (Badino, 2010; Mammola & Isaia, 2016). The facultative subterranean species can be assumed to be exposed to a maximum annual variation between 8 – 24.4º C (minimum temperature of the coldest month and maximum temperature of the warmest month at the surface in its locality, respectively, obtained from Worldclim v. 1.4 database (http://www.worldclim.org; see Table 1). Projected temperatures for 2070 under the Representative Concentration Pathway (RCP) 4.5 are also shown in Table 1.

Specimens of *S. crypticola* and *S. crotchi* were collected by hand in the caves indicated in Table 1, whilst specimens of *B. rugosa* were collected in forest litter near the entrances of Cave Orobe. The specimens were transported to the laboratory in a portable fridge with substratum from the cave and moss to retain humidity (ca. 90% RH). In the laboratory, they were maintained for two days prior to experiments in closed plastic containers (10 x 15 x 10 cm) with a plaster layer (1 cm), in controlled-temperature incubators (Radiber ERF-360, Radiber S.A, Barcelona, Spain) at

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the approximate mean temperature of their habitats (*S. crypticola* 11ºC; *S. crotchi* 9ºC; *B. rugosa* 12ºC), i.e. control temperatures hereafter. Maintenance of high humidity (> 90% RH) throughout the experiments was ensured by adding wetted tissue paper to experimental containers daily and placing trays with water inside the incubators. Temperature and humidity inside the containers were monitored with HOBO MX2301 dataloggers and remained quite constant, with very small variations (±0.5ºC and ±10% RH). Food was provided *ad libitum* with freshly frozen *Drosophila melanogaster*.

Upper thermal limits and acclimation capacity

Specimens were acclimated at either a control temperature (see above) or at 20ºC for 10 days (N = 10-13 individuals per treatment), with all other conditions (humidity, food etc.) as described before. These acclimation temperatures were chosen since all subterranean leiodids studied to date can survive relatively long-term exposure (>7 days) at 20ºC, but this is also close to their upper lethal limits (Rizzo *et al*., 2015; Pallarés *et al*., 2019, 2020), and it is well established that insects typically undergo acclimation in response to stressful sublethal temperatures (e.g. Lachenicht *et al*., 2010; Nyamukondiwa & Terblanche, 2010; Terblanche *et al*., 2011; Sgrò, Terblanche, & Hoffmann, 2016). After acclimation, we estimated heat coma temperature (HCT) for each individual. This was defined as the temperature at paralysis prior to death, preceded by spasmodic movements of legs and antennae, and is a typical response used to assess critical thermal maximum (CT_{max}) in insects (see Vannier, 1994; Lutterschmidt & Hutchison, 1997; Chown & Nicolson, 2004). HCT was measured using a dynamic, ramping method (Lutterschmidt & Hutchison, 1997; Terblanche *et al*., 2007), with a heating rate of 1ºC min^{-1} and infrared thermography. CT_{max} measured with such a fast ramping rate tend to be overestimated (Terblanche *et al*., 2007) and cannot be used to directly extrapolate actual thermal limits, but this method provides a robust comparative estimate of the relative thermal tolerance amongst related species and is the most commonly used in thermal tolerance assays on ectotherms (Bennett *et al.*,

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2018). Furthermore, infrared thermography has proven to be a highly sensitive technique to measure CT_{max} in insects (Botella-Cruz *et al*., 2016; Carbonell *et al*., 2016; Gallego *et al*., 2016; Gallego, Verdú, & Lobo, 2018). Specimens were dried on blotting paper and glued dorsally on a ceramic plate using nontoxic glue (ErichKrause) to prevent escape during the trial. The plate was then placed in a programmable BINDER MK53 incubator (BINDER GmbH, Tuttlingen, Germany) in which the temperature was increased 1ºC min^{-1}, starting at the corresponding acclimation temperature. The incubator was coupled with an infrared thermographic camera (FLIR SC305) to record body temperature of the specimens, from which HCT were obtained. High quality images were also recorded with a video camera (Sony DCR-DVD110E, Sony Co., Tokyo, Japan) to determine the moment of paralysis (cessation of movement of legs and antennae) of each individual. Thermal images were analysed with the software ThermaCAM Researcher Professional 2.10 (FLIR Advanced Thermal Solutions; ATS; Croissy-Beaubourg, France).

Statistical analyses

We performed a two-way ANOVA with HCT as the response variable and species, acclimation temperature (a factor with two levels: control temperature (see above) or 20ºC) and their interaction as predictors, to compare HCT and short-term acclimation capacity across species and assess whether these traits are related to the degree of subterranean specialisation. Pairwise comparisons were made with Bonferroni-adjusted post-hoc tests. Significance level was set at 0.05. Data conformed to a normal distribution so no transformation was required. Normality and homoscedasticity assumptions of model residuals were also validated by graphical inspection (Zuur *et al*., 2009). All statistical analyses were conducted in R v.3.6.1 (R Core Team, 2019) using default packages and *phia* (De Rosario-Martinez, 2015).

Results

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Heat coma temperatures (HCTs) ranged from 34.6 – 38ºC across the studied species and acclimation treatments (Figure 1). HCTs differed significantly amongst species (ANOVA $F_{2, 52}$ = 21.963, P < 0.001), being lower in the one-larval instar, deep subterranean species (*S. crypticola*) than in the other two, less specialised taxa investigated (all Bonferroni corrected Ps < 0.001, Table 2). There was also a significant effect of acclimation temperature on HCT (ANOVA $F_{1, 52}$ = 18.357, P < 0.001), but species differed significantly in their response to acclimation (ANOVA species x temperature interaction $F_{2, 52}$ = 3.564, P = 0.035).

The deep subterranean specialist, *S. crypticola*, had a significantly lower HCT than the two-instar, shallow subterranean *S. crotchi* when acclimated at control temperatures, and lower than either of the other two species following acclimation at 20ºC (all Bonferroni corrected Ps < 0.001, Table 2; Fig.1). Only the species with the least specialisation to subterranean environments, *B. rugosa* (three larval instars), showed acclimation capacity. In this species, HCT significantly increased after exposure at 20ºC (Bonferroni corrected P < 0.001, Table 2; Fig. 1), with a difference of 1.2ºC between the mean HCT of the control and 20ºC treatment. The more specialised subterranean taxa lacked acclimation ability because no differences were found in HCT between acclimation treatments (Bonferroni corrected Ps > 0.05, Table 2; Fig. 1).

Discussion

Among the three studied subterranean species, the most specialised to the subterranean environment and hence living under the more stable climatic conditions, *S. crypticola*, showed the lowest HCT values. Such finding in relation to the upper thermal limit fits the general predictions of the CVH, as seen in other subterranean groups for both lower (Latella *et al*., 2008; Lencioni *et al*., 2010; Novak *et al*., 2014) and upper thermal limits (Raschmanová *et al*., 2018; Mammola *et al*., 2019c). The main novelty of our study is that the CVH is tested by comparing not only thermal limits, but also thermal plasticity, something largely ignored for subterranean

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taxa to date. Again, in agreement with the CVH, the more specialised subterranean taxa (*S. crypticola* and *S. crotchi,* both obligate subterranean) lacked acclimation ability at the acclimation temperatures and heating rates tested here; whilst *B. rugosa*, the facultative subterranean species exposed to more fluctuating daily and seasonal temperatures, significantly increased HCT after acclimation at the higher temperature. As well as a positive acclimation response, *B. rugosa* showed the highest HCT values across the three species after acclimation at 20ºC. Therefore, there is not apparent evolutionary trade-off between CT_{max} and its short-term plasticity in these species, as reported in a clade of aquatic beetles (Calosi, Bilton & Spicer, 2008), but in contrast to patterns seen in other taxa such as intertidal porcellanid crabs (Stillman, 2003). As suggested by Calosi *et al*. (2008), the mechanisms and constraints underlying thermal limits and acclimation capacity appear to be taxon and context specific.

A lack of thermal acclimation capacity has also been reported for other subterranean groups, such as groundwater crustaceans (Di Lorenzo & Galassi, 2017), and previous work on Leptodirini suggests that the physiological mechanisms to cope with heat stress are rather inefficient in specialised subterranean species compared with their less specialised relatives. For example, Bernabò *et al.* (2011) showed that the intensity of the heat shock response was negatively correlated with the degree of adaptation to the cave environment. Pallarés *et al*. (2020) found that an obligate subterranean species of the clade studied here failed to acclimate when exposed to a fixed stressful temperature. Such inability to acclimate was coupled with oxidative stress and shifts in the activity of key enzymes at sublethal temperatures. The physiological mechanisms underlying thermal acclimation capacity in ectotherms are energetically costly processes (Krebs & Loeschcke, 1994; Monaghan, Metcalfe, & Torres, 2009; Tomanek, 2010) that could have been selected against during the evolution of specialisation to deep subterranean habitats. In these environments, with limited resources and highly stable climatic conditions, both temporally and spatially (Badino, 2010; Howarth & Moldovan, 2018),

traits that do not affect fitness could be loss or reduced either by neutral evolution or indirect natural selection favouring energetic economy (Culver & White, 2005, Ribera *et al*., 2018). Extreme modifications of thermal physiology have been found in organisms inhabiting other highly stable habitats, such as the absence of a heat shock response in Antarctic notothenioid fish (Somero, 2005). The greatest modifications of thermal physiology in subterranean taxa, resulting in lower thermal limits and poor acclimation capacity, may be expected in those lineages that colonised subterranean habitats longer ago during their evolutionary history. This is the case of Western Mediterranean Leptodirini, which were apparently already adapted to subterranean life by the Early-Mid Oligocene, some 34-28 MYA (Ribera *et al*., 2010; Cieslak, *et al*., 2014b). Accordingly, other specialised deep subterranean species of these beetles are also likely to lack acclimation capacity and have similar CT_{max} than the one-larval instar species studied here (*S. crypticola*). In contrast, deep subterranean species in lineages that colonised subterranean habitats more recently may retain greater heat tolerance and acclimation capacity from their less specialised or surface-dwelling ancestors, as previously suggested (Pallarés *et al*., 2019). Further experimental data on thermal limits and acclimation for several lineages that have independently colonised the subterranean environment would be invaluable in testing this evolutionary hypothesis. Unfortunately, the difficulties in accessing subterranean habitats, of collecting sufficient number of specimens for experimental studies and maintaining and rearing subterranean organisms in the laboratory (Raschmanová *et al*., 2018; Castaño-Sánchez *et al*., 2019; Mammola *et al*., 2019a) impose serious limitations when conducting comparative studies on cave-dwelling species. Therefore, despite its relatively modest taxonomic coverage, our study represents an important milestone in understanding physiological evolution of subterranean invertebrates.

Overall vulnerability to climate change in subterranean species will depend mainly on their thermal sensitivity and the magnitude and rate of climate change in their habitats, as range shifts

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through dispersal will be largely limited. Both aspects (sensitivity and exposure to climate change) will in turn be determined by the degree of subterranean specialisation, but in opposite directions. Here we show that subterranean species have higher thermal sensitivity than surface ones, something that has been generally assumed but rarely tested. The HCT of the three species studied are markedly lower than the average value of CT_{max} (45.5 ± 1.0ºC, N = 40) recorded experimentally in a range of arthropods at the same heating rate (Bennett *et al*., 2018). We also show that thermal sensitivity increases with subterranean specialisation. In deep subterranean Leptodirini, experiments under relatively long-term exposure, which may better represent natural conditions, have shown that heat injury at the biochemical level occurs at 20ºC (Pallarés *et al*., 2020) and survival is not possible above 23ºC (Rizzo *et al*., 2015). Such upper lethal limits seem to be highly conservative across deep subterranean specialist species of this clade, irrespective of the current and historical temperature of their habitats (Rizzo *et al*., 2015). Likewise, the lack of acclimation observed here in *S. crypticola* and *S. crotchi* appears to be mirrored in related species with similar specificity to subterranean habitats (as discussed above). In contrast, changes in the underground climate will be delayed compared to those experienced at the surface (Fejér & Moldovan, 2013; Domínguez-Villar *et al*., 2015) and will occur primarily in the cave sections closest to the surface and in superficial subterranean habitats (Culver & Pipan, 2014), such as small cavities in the uppermost karst layers, deep soil and litter strata, surface cracks and fissures (Badino, 2004; Mammola, Goodacre, & Isaia, 2018). In a proximate climate change scenario, the projected annual mean surface temperature apparently will not reach physiologically stressful levels for the two obligate subterranean species studied here (Table 1). However, this cannot be generalized, as some coastal or southern populations of specialist cave invertebrates in the Mediterranean Basin are currently living at temperatures close to their lethal limits (e.g. Sánchez-Fernández *et al*., 2016; Pallarés *et al*., 2019) and the same could occur in the tropics (Zeh *et al*., 2012). As the possibilities for behavioural thermoregulation through

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microhabitat selection are extremely reduced in the deep subterranean environment, and considering the limited capacity to physiologically adjust to rising temperature observed here, these habitats may become physiological and evolutionary traps for some of their obligate inhabitants (Mammola *et al*., 2019b). Compared to species confined to caves, those occupying forest litter or the MSS, like *B. rugosa,* may cope better with warming, because they are able to increase thermal limits via acclimation and exploit a wider range of microhabitats. In this case, the magnitude and rate of warming could be the key determinant of their vulnerability to climate change, as these species are more exposed to the thermal fluctuations of the surface, where maximum temperatures could reach physiologically stressful values in a proximate future (e.g. 28.5ºC in the localities where *B. rugosa* was collected).

In summary, our results stress the need to account for physiological constraints such as thermal sensitivity and acclimation capacity if we are to accurately forecast the impacts of global change on specialised invertebrates with low dispersal ability. This information, combined with evaluation of exposure to climatic changes in occupied locations, can greatly assist in identifying species or populations at higher risk. We show that in the case of the subterranean environment, the relative importance of factors determining overall vulnerability may differ depending on the degree of habitat specialisation. As many authors have already urged (e.g. Sánchez-Fernández *et al*., 2018; Mammola *et al*., 2019a,b; Castaño-Sánchez *et al.,* 2020), it is necessary to increase research, monitoring and conservation efforts on this unknown, fragile and valuable component of global biodiversity.

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References

Badino, G. (2004). Cave temperatures and global climatic change. *Int. J. Speleol*. **33**, 103–114.

Badino, G. (2010). Underground meteorology. What's the weather underground? *Acta Carsol*. **39**, 427-448.

Bennett, J.M., Calosi, P., Clusella-Trullas, S., Martínez, B., Sunday, J., Algar, A., Araújo, M.B., Hawkins, B.A., Keith, S., Kühn, I., Rahbek, C., Rodríguez, L., Singer, A., Villalobos, F., Angel Olalla-Tárraga, M. & Morales-Castilla, I. (2018). GlobTherm, a global database on thermal tolerances for aquatic and terrestrial organisms. *Sci. Data* **5**, 180022.

Bernabò, P., Latella, L., Jousson, O., & Lencioni, V. (2011). Cold stenothermal cave-dwelling beetles do have an HSP70 heat shock response. *J. Therm. Biol.* **36**, 206–208.

Botella-Cruz, M., Carbonell, J. A., Pallarés, S., Millán, A., & Velasco, J. (2016). Plasticity of thermal limits in the aquatic saline beetle *Enochrus politus* (Küster 1849) (Coleoptera: Hydrophilidae) under changing environmental conditions. *Limnetica* **35**, 131–142.

Calosi, P., Bilton, D. T., & Spicer, J. I. (2008). Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biol. Lett.* **4**, 99–102.

Carbonell, J. A., Millán, A., Green, A. J., Céspedes, V., Coccia, C., & Velasco, J. (2016). What traits underpin the successful establishment and spread of the invasive water bug *Trichocorixa verticalis verticalis*? *Hydrobiologia* **768**, 273–286. (doi:10.1007/s10750-015-2556-y)

Castaño-Sánchez, A., Hose, G. C., & Reboleira, A. S. P. S. (2020). Ecotoxicological effects of anthropogenic stressors in subterranean organisms: A review. *Chemosphere* **244**, 125422.

Chown, S. L., & Nicolson, S. W. (2004). *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford: Oxford University Press.

Cieslak, A., Fresneda, J., & Ribera, I. (2014a). Developmental constraints in cave beetles. *Biol. Lett.* **10**, 20140712.

Cieslak, A., Fresneda, J., & Ribera, I. (2014b). Life-history specialization was not an evolutionary dead-end in Pyrenean cave beetles. *Proc. R. Soc. B* **281**, 20132978.

Cigna, A. A. (2002). Modern trend(s) in cave monitoring. *Acta Carsol.* **31**, 35–54.

Cooke, S. J., & O'Connor, C. M. (2010). Making conservation physiology relevant to policy makers and conservation practitioners. *Cons. Lett.* **3**, 159-166.

Cooke, S. J., Sack, L., Franklin, C. E., Farrell, A. P., Beardall, J., Wikelski, M., & Chown, S. L. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conserv. Physiol.* **1**, cot001.

Covington, M.D., & Perne, M. (2015). Consider a cylindrical cave: A physicist's view of cave and karst science. *Acta Carsol*. **44**, 363–380.

Culver, D.C. & Pipan, T. (2014). *Shallow Subterranean Habitats. Ecology, Evolution, and Conservation*. Oxford: Oxford University Press.

Culver, D.C., & White, W.B. (2005). *Encyclopedia of caves*. Amsterdam: Elsevier.

Da Silva, C.R.B., Riginos, C., & Wilson, R.S. (2019). An intertidal fish shows thermal acclimation despite living in a rapidly fluctuating environment. *J. Comp. Physiol. B* **189**, 385-398.

De Rosario-Martinez, H. (2015). phia: Post-Hoc Interaction Analysis. R package version 0.2-1.

Review Copy

https://CRAN.R-project.org/package=phia

Di Lorenzo, T., & Galassi, D.M.P. (2017). Effect of temperature rising on the stygobitic crustacean species *Diacyclops belgicus*: Does global warming affect groundwater populations? *Water* **9**, 951.

Domínguez-Villar, D., Lojen, S., Krklec, K., Baker, A., & Fairchild, I. J. (2015). Is global warming affecting cave temperatures? Experimental and model data from a paradigmatic case study. *Clim. Dyn.* **45**, 569–581.

Evans, T. G., Diamond, S. E., & Kelly, M. W. (2015). Mechanistic species distribution modelling as a link between physiology and conservation. *Conserv. Physiol.* **3**, cov056.

Feder, M. E. (1978). Environmental variability and thermal acclimation in neotropical and temperate zone salamanders. *Physiol. Zool*. **51**, 7–16.

Fejér, A., & Moldovan, O. (2013). Population size and dispersal patterns for a *Drimeotus* (Coleoptera, Leiodidae, Leptodirini) cave population. *Subt. Biol*. **11**, 31–44. (doi:10.3897/subtbiol.11.4974)

Foden, W.B., Young, B.E., Akçakaya, H.R., Garcia, R.A., Hoffmann, A. A., Stein, B.A., *et al*. (2019) .Climate change vulnerability assessment of species. *Wiley Interdiscip. Rev. Clim. Change* **10**, e551.

Gallego, B., Verdú, J. R., & Lobo, J. M. (2018). Comparative thermoregulation between different species of dung beetles (Coleoptera: Geotrupinae). *J. Therm. Biol.* **74**, 84–91.

Gallego, B., Verdú, J. R., Carrascal, L. M., & Lobo, J. M. (2016). A protocol for analysing thermal stress in insects using infrared thermography. *J. Therm. Biol.* **56**, 113–121.

Gaston, K. J., Chown, S. L., Calosi, P., Bernardo, J., Bilton, D. T., Clarke, A., Clusella-Trullas, S., Ghalambor, C. K., Konarzewski, M., Peck, L. S., Porter, W. P., Pörtner, H. O., Rezende,

Review Copy

E. L., Schulte, P. M., Spicer, J. I., Stillman, J. H., Terblanche, J. S., & van Kleunen, M. (2009). Macrophysiology: A Conceptual Reunification. *Amer. Nat*. **174**, 595–612.

Glaçon, S. (1955). Remarques sur la morphologie et la biologie de quelques larves de Bathysciinae cavernicoles. *CR Acad. Sci. Paris* **240**, 679-681.

Howarth, F. G., & Moldovan, O. T. (2018). Where Cave Animals Live. In *Cave Ecology:* 23–37. Moldovan, O.T., Kovác, L., & Halse, S. (Eds.) Cham: Springer.

Issartel, J., Hervant, F., Voituron, Y., Renault, D., & Vernon, P. (2005). Behavioural, ventilatory and respiratory responses of epigean and hypogean crustaceans to different temperatures. Comp. Biochem. Phys. A **141**, 1-7.

Jeannel, R. (1911). Rèvision des Bathysciinae. *Arch. Zool. Exp. Gen. Paris Ser 5*. **7**, 1–641.

Juan, C., & Emerson, B.C. (2010). Evolution underground: shedding light on the diversification of subterranean insects. *J. Biol.* ***9***, 17.

Juan, C., Guzik, M.T., Jaume, D. & Cooper, S.J.B. (2010). Evolution in caves: Darwin's 'wrecks of ancient life' in the molecular era. *Mol. Ecol.* **19**, 3865–3880.

Juberthie, C., Delay, B., & Bouillon, M. (1980a). Extension du milieu souterrain superficiel en zone non-calcaire: description d'un nouveau milieu et de son peuplement par les coleopteres troglobies. In *Evolution des coleopteres souterrains et endoges*. *Mem. Biospeol*. **7**, 19–52.

Juberthie, C., Delay, B., & Bouillon, M. (1980b). Sur l'existence d'un milieu souterrain superficiel en zone non calcaire. *CR Acad. Sci. Paris* **290**, 49–52.

Juberthie, C., Delay, B., & Bouillon, M. (1981). Sur l'existence du milieu souterrain superficiel en zone calcaire. In *Les entrees d'energie dans le karst et communications libres*. *Mem. Biospeol.* **8**, 77–93.

Krebs, R. A., & Loeschcke, V. (1994). Costs and benefits of activation of the heat-shock response

in *Drosophila melanogaster*. *Funct. Ecol.* **8**, 730–737.

Lachenicht, M. W., Clusella-Trullas, S., Boardman, L., Le Roux, C., & Terblanche, J. S. (2010). Effects of acclimation temperature on thermal tolerance, locomotion performance and respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). *J. Insect Physiol.* **56**, 822–830.

Latella, L., Bernabò, P., & Lencioni, V. (2008). Distribution pattern and thermal tolerance in two cave-dwelling Leptodirinae (Coleoptera, Cholevidae). *Subterr. Biol.* **6**, 81–86.

Lencioni, V., Bernabò, P., & Latella, L. (2010). Cold resistance in two species of cave-dwelling beetles (Coleoptera: Cholevidae). *J. Therm. Biol*. **35**, 354-359.

Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Can. J. Zool.* **75**, 1553–1560.

Magozzi, S., & Calosi, P. (2015). Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Glob. Chang. Biol.* **21**, 181–194.

Mammola, S., & Isaia, M. (2016). The ecological niche of a specialized subterranean spider. *Invertebr. Biol*. **135**, 20–30.

Mammola, S., Cardoso, P., Culver, D. C., Deharveng, L., Ferreira, R. L., Fišer, C., Galassi, D. M. P., Griebler, C., Halse, S., Humphreys, W. F., Isaia, M., Malard, F., Martinez, A., Moldovan, O. T., Niemiller, M. L., Pavlek, M., Ana Sofia P, Souza-Silva, M., Teeling, E. C., Judson Wynne, J., & Zagmajster, M. (2019a). Scientists' Warning on the Conservation of Subterranean Ecosystems. *BioScience* **69**, 641–650

Mammola, S., Giachino, P. M., Piano, E., Jones, A., Barberis, M., Badino, G., & Isaia, M. (2016). Ecology and sampling techniques of an understudied subterranean habitat: the Milieu Souterrain Superficiel (MSS). *Naturwissenschaften* **103**, 88.

ACV submitted manuscript

Review Copy

Mammola, S., Goodacre, S. L., & Isaia, M. (2018). Climate change may drive cave spiders to extinction. *Ecography* **41**, 233–243.

Mammola, S., Piano, E., Cardoso, P., Vernon, P., Domínguez-Villar, D., Culver, D. C., Pipan, T., & Isaia, M. (2019b). Climate change going deep: The effects of global climatic alterations on cave ecosystems. *Anthr. Rev*. **6**, 98–116.

Mammola, S., Piano, E., Malard, F., Vernon, P., & Isaia, M. (2019c). Extending Janzen's hypothesis to temperate regions: A test using subterranean ecosystems. *Funct. Ecol.* **33**, 1638–1650.

Markle, T. M., & Kozak, K. H. (2018). Low acclimation capacity of narrow-ranging thermal specialists exposes susceptibility to global climate change. *Ecol. Evol.* **8**, 4644–4656.

Mermillod-Blondin, F., Lefour, C., Lalouette, L., Renault, D., Malard, F., Simon, L., & Douady, C. J. (2013). Thermal tolerance breadths among groundwater crustaceans living in a thermally constant environment. *J. Exp. Biol.* **216**, 1683-1694.

Minelli, A., & Fusco, G. (2013). Arthropod post-embryonic development. In *Arthropod Biology and Evolution*: 91–122. Minelli, A., Boxshall, G., & Fusco, G. (Eds.) Berlin: Springer.

Monaghan, P., Metcalfe, N. B., & Torres, R. (2009). Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol. Lett.* **12**, 75–92.

Moore, G. W., & Sullivan, G. N. (1964). Out of phase seasonal temperature fluctuations in Cathedral Cave, Kentucky. *Geol. Soc. Am*. **76**, 313

Novak, T., Šajna, N., Antolinc, E., Lipovšek, S., Devetak, D., & Janžekovič, F. (2014). Cold tolerance in terrestrial invertebrates inhabiting subterranean habitats. *Int. J. Speleol*. **43**, 3.

Nyamukondiwa, C., & Terblanche, J. S. (2010). Within-generation variation of critical thermal limits in adult Mediterranean and Natal fruit flies *Ceratitis capitata* and *Ceratitis ros*a:

thermal history affects short-term responses to temperature. *Physiol. Entomol*. **35**, 255–264.

Pallarés, S., Colado, R., Pérez-Fernández, T., Wesener, T., Ribera, I., & Sánchez-Fernández, D. (2019). Heat tolerance and acclimation capacity in subterranean arthropods living under common and stable thermal conditions. *Ecol. Evol.* **9**, 13731–13739.

Pallarés, S., Sanchez-Hernandez, J.C., Colado, R., Balart-García, P., Comas, J. & Sánchez-Fernández, D. (2020). Beyond survival experiments: using biomarkers of oxidative stress and neurotoxicity to assess vulnerability of subterranean fauna to climate change. *Conserv. Physiol.* **8**, coaa067.

Pipan, T., López, H., Oromí, P., Polak, S., & Culver, D. C. (2010). Temperature variation and the presence of troglobionts in terrestrial shallow subterranean habitats. *J. Nat. Hist*. **45**, 257–273.

Pipan, T., Petrič, M., Šebela, S. & Culver, D.C. (2018). Analyzing climate change and surface–subsurface interactions using the Postojna Planina Cave System (Slovenia) as a model system. *Reg. Environ. Change* **19**, 379–389.

Pipan, T., Petrič, M., Šebela, S., & Culver, D.C. (2018). Analyzing climate change and surface–subsurface interactions using the Postojna Planina Cave System (Slovenia) as a model system. *Reg. Environ. Change* **19**, 379–389.

R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raschmanová, N., Šustr, V., Kováč, Ľ., Parimuchová, A., & Devetter, M. (2018). Testing the climatic variability hypothesis in edaphic and subterranean Collembola (Hexapoda). *J. Therm. Biol*. **78**, 391–400.

Ribera, I., Cieslak, A., Faille, A., & Fresneda, J. (2018). Historical and ecological factors determining cave diversity. In *Cave Ecology*: 229-252. Moldovan, O.T., Kovác, L., & Halse,

Review Copy

S. (Eds.) Cham: Springer.

Ribera, I., Fresneda, J., Bucur, R., Izquierdo, A., Vogler, A. P., Salgado, J. M., & Cieslak, A. (2010). Ancient origin of a Western Mediterranean radiation of subterranean beetles. *BMC Evol. Biol.* **10**, 29.

Rizzo, V., Sánchez-Fernández, D., Alonso, R., Pastor, J., & Ribera, I. (2017). Substratum karstificability, dispersal and genetic structure in a strictly subterranean beetle. *J. Biogeogr.* **44**, 2527-2538.

Rizzo, V., Sánchez-Fernández, D., Fresneda, J., Cieslak, A., & Ribera, I. (2015). Lack of evolutionary adjustment to ambient temperature in highly specialized cave beetles. *BMC Evol. Biol.* **15**, 10.

Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecol. Lett.* **21**, 1425–1439.

Salgado, J. M., Blas, M., & Fresneda, J. (2008). *Fauna Ibérica, Vol. 31, Coleoptera, Cholevidae*. Madrid: MNCN, CSIC.

Sánchez-Fernández, D., Rizzo, V., Bourdeau, C., Cieslak, A., Comas, J., Faille, A., Fresneda, J., Lleopart, E., Millán, A., Montes, A., Pallarés, S., & Ribera, I. (2018). The deep subterranean environment as a potential model system in ecological, biogeographical and evolutionary research. *Subt. Biol*. **25**, 1.

Sánchez-Fernández, D., Rizzo, V., Cieslak, A., Faille, A., Fresneda, J., & Ribera, I. (2016). Thermal niche estimators and the capability of poor dispersal species to cope with climate change. *Sci. Rep*. **6**, 23381.

Šebela, S., Turk, J., & Pipan, T. (2015). Cave micro-climate and tourism: Towards 200 years (1819–2015) at Postojnska jama (Slovenia). *Cave & Karst Science* **42**, 78–85.

Review Copy

Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nat. Clim. Change* **5**, 61-66.

Sgrò, C. M., Terblanche, J. S., & Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* **61**, 433–451.

Shah, A. A., Funk, W. C., & Ghalambor, C. K. (2017). Thermal acclimation ability varies in temperate and tropical aquatic insects from different elevations. *Integr. Comp. Biol.* **57**, 977–987.

Sket, B. (2008). Can we agree on an ecological classification of subterranean animals? *J. Nat. Hist.* **42**, 1549–1563.

Smithson, P. A. (1991). Inter-relationships between cave and outside air temperatures. *Theor. Appl. Climatol*. **44**, 65–73.

Somero, G. N. (2005). Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Front. Zool.* **2**, 1.

Stevens, G. C. (1989). The latitudinal gradient in geographical range: how so many species coexist in the tropics. *Amer. Naturalist* **133**, 240–256.

Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65.

Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C., & Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proc. Biol. Sci.* **274**, 2935–2942.

Terblanche, J. S., Hoffmann, A. A., Mitchell, K. A., Rako, L., le Roux, P. C., & Chown, S. L. (2011). Ecologically relevant measures of tolerance to potentially lethal temperatures. *J. Exp. Biol.* **214**, 3713–3725.

Tomanek, L. (2008). The importance of physiological limits in determining biogeographical

Review Copy

range shifts due to global climate change: the heat-shock response. *Physiol. Biochem. Zool.* **81**, 709–717.

Tomanek, L. (2010). Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. *J. Exp. Biol.* **213**, 971–979.

Trontelj, P., Borko, Š., & Delić, T. (2019). Testing the uniqueness of deep terrestrial life. *Sci. Rep.* **9**, 15188.

Vannier, G. (1994). The thermobiological limits of some freezing intolerant insects: the supercooling and thermostupor points. *Acta Oecol.* **15**, 31–42.

Voituron, Y., De Fraipont, M., Issartel, J., Guillaume, O. & Clobert, J. (2010). Extreme lifespan of the human fish (*Proteus anguinus*): A challenge for ageing mechanisms. *Biol. Lett.* **7**, 105–107.

Wikelski, M., & Cooke, S.J. (2006). Conservation physiology. *Trends Ecol. Evol*. 21, 38-46.

Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A., & Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol.* **6**, e325.

Zeh, J.A., Bonilla, M.M., Su, E.J., Padua, M.V., Anderson, R.V., Kaur, D., Yang, D. & Zeh, D.W. (2012). Degrees of disruption: projected temperature increase has catastrophic consequences for reproduction in a tropical ectotherm. *Glob. Chang. Biol.* **18**, 1833-1842.

Zuur, A., Ieno, E., Walker, N., Saveliev, A., & Smith, G. (2009). Mixed effects models and extensions in ecology with R. New York: Springer

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Tables

Table 1. Information on collection sites and their current and projected temperature.

Species	No instars	Locality	Province	Mean annual Temperature (ºC)[a]		Annual Temperature range (ºC)[a,b]	
				Current	**2070**	**Current**	**2070**
Speonomites crypticola	1	Cave Forat Negre	Lleida	10.4	11.8	-2 – 23.1	-0.8 – 28.0
Speonomidius crotchi	2	Cave Arrikrutz	Guipuzcoa	11.1	14.9	3.4 – 24.6	5.2 - 28.4
Bathysciola rugosa	3	Cave Orobe	Navarra	12	13.6	8 – 24.4	26.8 – 28.5

[a]Source: Worldclim v. 1.4 database (https://www.worldclim.org/), 30 second spatial resolution.

Future projected temperatures in 2070 were obtained for the Representative Concentration Pathway 4.5 and by averaging 17 different Global Circulation Models.

[b]Minimum temperature of the coldest month – Maximum temperature of the warmest month

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Table 2. Results of post-hoc tests (with Bonferroni-adjusted P-values) to compare heat coma temperature i) among species, ii) between acclimation treatments (C: control vs 20ºC) within each species and iii) among species within each acclimation treatment.

	Value	**df**	**Sum of Sq**	**F**	**P**
B. rugosa - *S. crotchi*	-0.088	1	0.073	0.220	1
B. rugosa - *S. crypticola*	1.047	1	8.698	26.125	<0.001
S. crotchi - *S. crypticola*	1.135	1	13.206	39.668	<0.001
Residuals		52	17.312		
B. rugosa (C - 20)	-1.336	1	6.643	19.953	<0.001
S. crotchi (C - 20)	-0.446	1	1.294	3.886	0.162
S. crypticola (C - 20)	-0.360	1	0.548	1.646	0.615
Residuals		52	17.312		
C (*B. rugosa* - *S. crotchi*)	-0.532	1	1.400	4.206	0.272
C (*B. rugosa* - *S. crypticola*)	0.560	1	1.327	3.985	0.307
C (*S. crotchi* - *S. crypticola*)	1.091	1	6.335	19.030	<0.001
20 (*B. rugosa* - *S. crotchi*)	0.356	1	0.577	1.732	1
20 (*B. rugosa* - *S. crypticola*)	1.534	1	8.784	26.385	<0.001
20 (*S. crotchi* - *S. crypticola*)	1.178	1	6.871	20.638	<0.001
Residuals		52	17.312		

df: degrees of freedom, Sum of Sq: sum of squares

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Figure legends

Figure 1. Mean ± s.e.m heat coma temperatures measured after acclimation at a control temperature (*S. crypticola* 11ºC; *S. crotchi* 9ºC; *B. rugosa* 12ºC) or 20ºC. Significant differences between acclimation treatments within each species (P<0.05 in post-hoc tests) are indicated with asterisks.

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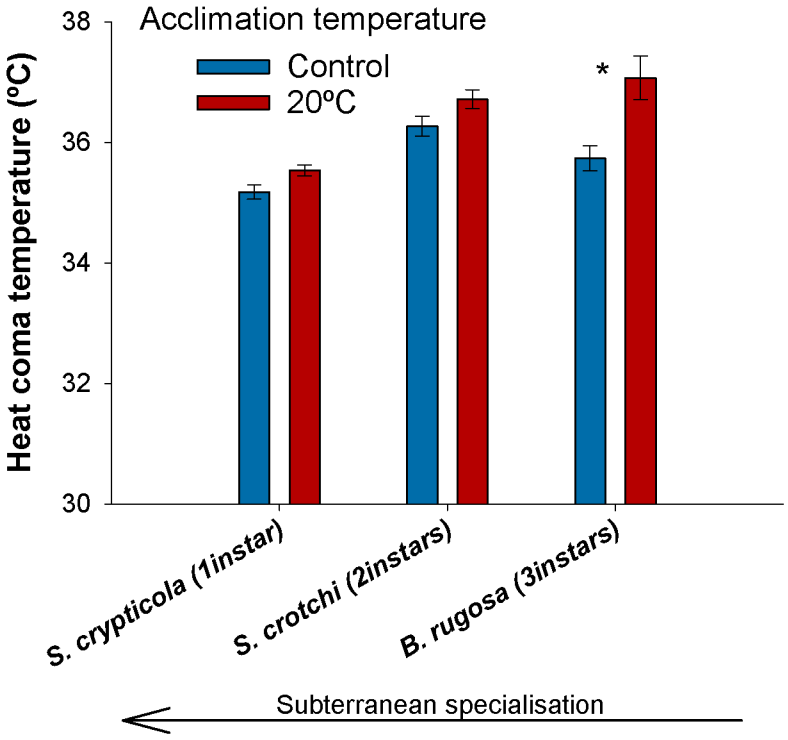
Figures


Acclimation temperature
Control
20ºC
Heat coma temperature (ºC)
38
36
34
32
30
*
S. crypticola (1instar)
S. crotchi (2instars)
B. rugosa (3instars)
Subterranean specialisation