Thermal tolerance and vulnerability to climate change in subterranean species: a case study using an Iberian endemic pseudoscorpion

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Abstract. 1. Scientists are renewing their efforts to predict the impact of climate change on biodiversity. Subterranean environments represent ideal systems to study the effect of global change in species with poor dispersal capabilities.

2. We assess the vulnerability to climate change of the subterranean pseudoscorpion *Neobisium (Blothrus) vasconicum vasconicum* (Nonídez, 1925) (Neobisiidae).

3. Thermal tolerance was measured using two complementary estimates of upper thermal limits: (i) from thermal conditions of the localities in which the species occurs (realised upper thermal limit, RUTL), and (ii) from experimentally determined thermal tolerance data (physiological upper thermal limit, PhUTL). Then, thermal safety margins (TSM) were calculated for all known localities for current and future climatic conditions, using the thermal limits from both approaches.

4. The physiological thermal limit (PhUTL = 17.57°C) was 3.27°C higher than that obtained from the distributional and climate data (i.e., the hottest cave in which the species occurs; RUTL = 14.3°C). Regarding TSM, the future temperature (2070; RCP 8.5) of a half of the caves will be higher than the RUTL and in none of them, it would exceed the average PhUTL. This indicates that the species could have some physiological capacity to cope with warming temperatures *in situ*.

5. We hypothesize that the most realistic upper thermal limit of the species could be between the RUTL and PhUTL. This study shows that complementary approaches to estimate thermal tolerance could provide more accurate predictions of the capacity to face climate change, not only in subterranean species, but also in poor dispersal species.

Key words. Climate data, distributional data, global change, physiological tolerance, species distribution models, subterranean biodiversity, thermal safety margin, upper thermal limit.

Introduction

In the context of climate change, a key challenge in ecology and conservation biology is to predict how species will respond to environmental changes (Bellard *et al.*, 2012; Román-Palacios & Wiens, 2020). If we aim to anticipate these changes and develop effective management strategies, accurate predictions of species response to climate change are essential (Grimm *et al.*, 2020; Samways *et al.*, 2020).

The vulnerability of a species to global warming depends on its capacity to (i) maintain present populations, which is ultimately related to its ecological niche breadth; and (ii) shift its geographical range to suitable environments, which depends not only on the amount and location of the available habitat in the future, but also on its dispersal capacity (Williams *et al.*,

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2008; Arribas *et al.*, 2017). However, most studies evaluating species vulnerability to climate change have focused exclusively on the expected changes in habitat availability under future climatic scenarios using correlative species distribution models (SDMs). While these approaches could be informative for species that are close to climate-equilibrium, that is, for well-dispersal species whose distributions are mainly determined by environmental variables (Pearson & Dawson, 2003; Sánchez-Fernández *et al.*, 2012), their utility for narrow-range poor-dispersal species is currently questioned (Jiménez-Valverde *et al.*, 2008; Sánchez-Fernández *et al.*, 2016; but see Mammola & Leroy, 2018). For these species, assessments of global warming impacts based exclusively on SDMs could provide inaccurate predictions, frequently underestimating the persistence of species *in situ* and overestimating their potential to access and exploit predicted future climate space (Guisan & Thuiller, 2005; Elith & Leathwick, 2009). Moreover, these approaches are often limited by the difficulty to account for the complexity of environmental factors that actually influence organisms and for biotic interactions, adaptive evolution or behavioural adjustments to highly heterogeneous habitats (Lavergne *et al.*, 2010; Hannah *et al.*, 2014; Sunday *et al.*, 2014).

In nature, there are few habitats in which the uncertainties exposed above are minimised, as for example, the subterranean environment, whose stable climatic conditions can be relatively easy to reproduce under controlled laboratory conditions. In contrast to what happens in surface environments, the variability of environmental conditions affecting a species underground is minimal. The humidity is always near the saturation point, and the temperature is highly constant through the day, season and year, and interestingly, it is established that it is approximately equal to the mean annual temperature at the surface (Poulson & White, 1969; Culver & Pipan, 2009; Sánchez-Fernández *et al.*, 2018). In addition, environmental conditions are highly homogeneous across all microhabitats within a cave system, compared to most superficial environments, so the possibility of avoiding stress through behavioural adjustments are minimal (Mammola *et al.*, 2019b). Also, caves harbour very simple biological communities (Culver & Pipan, 2009), which minimises the complexity of accounting for biological interactions. Furthermore, most highly specialised cave species also show low dispersal capacity imposed by the geological setting and extremely narrow and isolated geographical ranges (e.g., Cardoso, 2012; Niemiller *et al.*, 2013; Fattorini *et al.*, 2016; Rizzo *et al.*, 2017). Persistence in their current localities is therefore critical for subterranean species to face climate change, as dispersal to more suitable habitats is not an option. In sum, unlike superficial environments, in subterranean environments, it is easy to have an accurate knowledge about the actual environmental conditions that species experience. Therefore, these particular natural laboratories and their inhabitants are ideal model systems to study the effects of global change on species with poor dispersal capabilities as well as testing biogeographical, ecological and evolutionary hypotheses (Sánchez-Fernández *et al.*, 2018; Mammola, 2019).

Changes in the underground climate will be delayed compared to those experienced at the surface (Fejér & Moldovan, 2013; Domínguez-Villar *et al.*, 2015) affecting mainly in the cave sections closest to the surface and in superficial subterranean habitats (Culver & Pipan, 2014). Since warming-induced stress is the most proximate effect of global warming, and given the unprecedented rapid rates of temperature increase, species upper thermal tolerance could be the most immediate determinant of the effects of climate change (Bernardo & Spotila, 2006; Helmuth, 2009), preceding evolutionary responses (Chown *et al.*, 2004; Pörtner & Farrell, 2008). While research on thermal tolerance has been well developed for a wide range of taxa, from marine invertebrates to mammals (Bozinovic *et al.*, 2011), it is still at a very early stage in subterranean fauna (Castaño-Sánchez *et al.*, 2020b). The few studies on this field have shown that subterranean species have lower tolerance to high temperatures than epigean (surface) taxa, some of them being extremely sensitive to thermal changes (e.g.: Issartel *et al.*, 2005; Mermillod-Blondin *et al.*, 2013; Rizzo *et al.*, 2015; Sánchez-Fernández *et al.*, 2016; Raschmanová *et al.*, 2018; Mammola *et al.*, 2019c, Pallarés *et al.*, 2019). However, for some invertebrate groups well represented in the subterranean environment, physiological studies of thermal tolerance are scarce (Castaño-Sánchez *et al.*, 2020b) or completely lacking. This is the case of pseudoscorpions, with over 300 cave-adapted species in temperate and tropical areas (Howarth, 2009).

The aim of this study is to assess the vulnerability to climate change of the subterranean pseudoscorpion *Neobisium (Blothrus) vasconicum vasconicum* (Nonídez, 1925) (Neobisiidae), an Iberian endemism only known from a few caves in the North of Spain. As temperature is likely to be the most significant environmental variable among the few ones that might affect this species in the constant and homogeneous cave environment, we used two approaches to estimate its thermal tolerance, concretely its upper thermal limit (UTL). First, we used a traditional approach based on the thermal conditions of the localities in which the species has been found to estimate the realised upper thermal limit (RUTL). We then estimated the physiological upper thermal limit (PhUTL) from experimentally determined thermal tolerance data. Finally, thermal safety margins (TSM; Deutsch *et al.*, 2008) were calculated for each population using the thermal limits obtained from both approaches and considering both current and future climatic conditions.

Methods

Target species

We focus here on *Neobisium (Blothrus) vasconicum vasconicum* (Nonídez, 1925), a subterranean pseudoscorpion, endemic of the Gipuzkoa region (North of the Iberian Peninsula; see Fig. 1), described from the Mendikute cave (Ernio Massif) (Table 1). It is a predator, obligate-subterranean (troglobiont) species, restricted to the deep subterranean environment, with pronounced adaptations to live underground (i.e., troglomorphisms), such as exaggerated development of pedipalps, large body size, and depigmentation (Zaragoza & Galan, 2007).
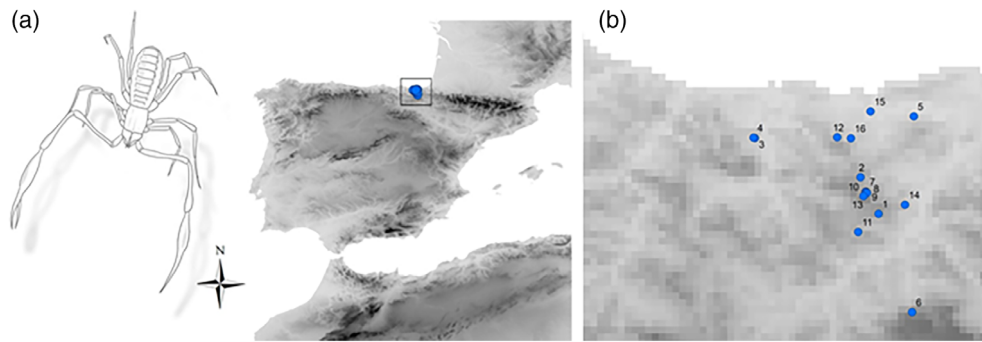
Fig 1. Study area. (a) Distribution range of *Neobisium vasconicum vasconicum*; (b) Blue points represent the 16 caves with known populations of this species. The greyscale of the background represents a gradient of altitude (see codes in Table 1).

Taxonomic validation

A bibliographic review was carried out to know the pseudoscorpion species present in the area where the specimens were collected (Guipuzkoa region, North of Spain; see Table 1) (Zaragoza, 2007), photographs were sent to experts (included in the acknowledgments section) and some specimens were examined under a microscope to confirm the taxonomic characters of the species, following identification keys (Zaragoza Miralles, 2000). The current nomenclature of the taxon was validated by reviewing the specific bibliography of this animal group in the Iberian Peninsula (Zaragoza, 2017). The collected specimens remain in the collection of the aquatic ecology research group, at the Faculty of Biology of the University of Murcia (Spain).

Estimating realised upper thermal limit from distribution and climatic data

To obtain an updated distribution of this species, we compiled all georeferenced localities from an exhaustive bibliographic review

(Zaragoza, 2007; Zaragoza & Galan, 2007) and other unpublished information (J.A. Zaragoza and C. Galán, pers. comm.).

Cave temperature can be estimated (though approximately) from the mean annual temperature of the surface (Culver & Pipan, 2009; Sánchez-Fernández *et al.*, 2018). These values were obtained from WORLDCLIM (version 1.4, http://www.worldclim.org; for details, see Hijmans *et al.*, 2005) at a spatial resolution of 30 arc-s (approximately 1 × 1-km resolution). The temperature estimated from the hottest cave in which the species occurs was considered as the "realized upper thermal limit" (RUTL), in other words, this value corresponds to the maximum temperature at which the species is exposed in nature.

Complementarily, we estimated the species' potential distribution using a simple thermal envelope procedure, as this approach allowed us to represent regions with favourable climatic conditions based on observed occurrences (see Jiménez-Valverde *et al.*, 2008; Lobo *et al.*, 2010; Soberón, 2010 for the conceptual basis of this approach). We used this approach to be conservative, as it is an established procedure aimed at maximising the potential distribution of species when only distributional data are used (Jiménez-Valverde *et al.*, 2011). We

Table 1. Caves with confirmed presence of *Neobisium vasconicum vasconicum*.

Cave code	Name	Massif	Latitude	Longitude	Altitude
1	Mendikute'kokoba	Ernio	43.145814	−2.123784	710
2	Sagainzelaia	Ernio	43.190122	−2.145745	670
3	Ekain 1	Izarraitz	43.23787	−2.274325	90
4	Ekain 2	Izarraitz	43.238322	−2.276105	111
5	Guardetxe'koleizea	Ernio	43.264519	−2.080806	140
6	Marizulo	Aralar	43.025864	−2.082939	1115
7	Leizeaundia 1	Ernio	43.172721	−2.13927	720
8	Leizeaundia 2	Ernio	43.172497	−2.139396	720
9	Sabesaia'koleizea	Ernio	43.171504	−2.139164	750
10	Sabesaia'kokoba	Ernio	43.171044	−2.137818	720
11	Isetxe'kokobea	Ernio	43.123443	−2.148444	450
12	Pagoeta'kokoba	Ernio	43.238758	−2.174182	465
13	Mako'koleizea	Ernio	43.167115	−2.142178	1010
14	Aizkoate	Ernio	43.156818	−2.091401	425
15	Altxerrikokoba	Ernio	43.270423	−2.133459	25
16	ZazpiIturri	Ernio	43.23760	−2.15739	375

calculated the maximum and minimum scores (extreme values) for mean annual temperature across all observed localities. Thus, we selected as suitable those areas with climatic values falling within that range and designating as unsuitable all areas outside it. These extreme values were used to derive a simple binary distributional hypothesis about the areas having climatically suitable conditions (potential distribution), assuming that recorded occurrences reflect the full spectrum of climatic conditions in which the species can survive and reproduce.

Estimating upper thermal limit from physiological experiments

Live adult specimens were collected by hand at the Mendikute cave (see Table 1) in February 2020. Once collected, specimens were placed in a portable fridge (adjustable temperature) with moss to maintain humid (>90% relative humidity) and darkness to minimise stress during transport.

In the laboratory (University of Castilla-La Mancha, Toledo, Spain), specimens were placed inside plastic boxes with a white plaster (Velox®, Ernst Hinrichs) layer (approximately 1 cm), moss, small stones and tissue papers that were wetted daily along the experiment to keep high humidity conditions. The boxes were closed with plastic film with small holes for aeration. Individuals were fed *ad libitum* with freshly frozen *Drosophila melanogaster* and maintained for 3 days before the experiments in an incubator (Radiber ERF-360: Radiber, Barcelona, Spain) at 10°C, the approximate temperature of the collection cave, for habituation to laboratory conditions. Humidity was kept near saturation (>90% RH) by placing trays with water in the incubators. Temperature and humidity were monitored every 15′ with dataloggers (HOBO MX2301, Onset Computer Corporation, Bourne, MA, USA and TFA 30.3039, KlimaLogg Pro: TFA, Wertheim-Reicholzheim, Germany) and remained highly constant (±0.5°C and ±10% RH variation) along the experiments.

To determine the upper thermal limit from physiological experiments (PhUTL) of our species, survival was measured at four different temperatures, which represent its current natural conditions (10°C, used as a control treatment), and potentially sublethal (20 and 23°C) or extreme temperatures (25°C) according to the previous tolerance limits reported for other highly specialist in subterranean species within the same study area (i.e., between 20 and 23°C) (Rizzo *et al.*, 2015; Pallarés *et al.*, 2020). A static method was used (Lutterschmidt & Hutchison, 1997; Jørgensen *et al.*, 2019), measuring survival under exposure at these constant temperatures for 7 days. Specimens (7–8 individuals per treatment) were placed in the plastic containers in the incubators at different test temperatures, with constant RH (>90%), permanent darkness and food *ad libitum*. Survival was checked every 24 h by observing specimens' motions after carefully touching them with a brush.

Survival at the different tested temperatures along the 7 days exposure was compared using Kaplan–Meier survivorship curves (Altman, 1990). To assess the overall effect of temperature treatment on survival time by a log-rank test (Harrington & Fleming, 1982), the R package *survival* v. 3.1–12 was used and the LT_{50} value as an estimation of physiological heat tolerance limit (PhUTL). This value represents the temperature at which 50%

of the exposed individuals have died at 7 days and it was estimated by fitting survival data to a logistic regression model using the R package *brglm* v. 0.6.2 and the *dose.p* function (package MASS v. 7.3–51.5). All statistical analyses were conducted using R 3.6.3.

Thermal safety margins

TSM were estimated for each locality as the difference between the estimated temperature of the locality for both current and future scenarios and the estimated thermal limits, considering both RUTL (TSM_{RUTL}) and PhUTL (TSM_{PhUTL}). Data on estimated future climatic conditions (annual mean temperature), were obtained from WORLDCLIM (version 1.4, see Hijmans *et al.*, 2005) at the same resolution that current temperature (i.e., approximately 1 × km grid cells), considering two RCP (representative concentration pathways) or different concentration carbon emission careers: 4.5 and 8.5 and two temporal scenarios (2050 and 2070).

Results

Realised upper thermal limits (RUTL)

The known distribution of our model species is restricted to only 16 caves located in the North of Spain (see Table 1 and Fig. 1). The temperature of these caves ranges from 10.2 (Marizulo) to 14.3°C (Altxerrikokoba). Thus, 14.3°C was considered as the RUTL. According to such limit, most of the study area would be habitable for the species under current climatic conditions (Table 2; Fig. S1a). However, most of this area would become unsuitable under future climatic conditions (Table 2; Fig S1b-e).

Physiological upper thermal limits

Temperature had a significant effect on survival time (log–rank test: $P < 0.001$, Table S1). The species showed approximately 90 and 50% survival at 10°C (cave temperature) and 20°C, respectively, during the 7 days of the experiment. Exposure to 23°C caused a progressive decrease of survival, which led to almost 100% mortality at the end of the experiment. No specimens survived longer than 1 day at 25°C (Fig. 2). The estimated LT_{50} at 7 days, that is, the physiological thermal limit (PhUTL), was 17.57 ± 1.93, considerably higher (3.3°C) than the upper thermal limit obtained from the hottest cave in which the species occurs (RUTL).

Thermal safety margins (TSM)

For all occurrence localities, the average TSM_{PhUTL} was significantly higher than TSM_{RUTL} for both current (4.74 ± 1.33 vs. 2.05 ± 1.37, respectively) and future climatic conditions (2.54 ± 1.31 vs. 0.91 ± 1.36, respectively, RCP 8.5 for 2070) (Table 2; Fig. 3). Indeed, while the future temperature (2070;

Table 2. Mean annual temperature (MAT, °C) and thermal safety margins (TSM, °C) for each locality calculated using realised upper thermal limit (TSM$_{RUTL}$) and physiological thermal limit (TSM$_{PhUTL}$) for current and future scenarios (RCP: Representative concentration pathways).

Cave code	MAT[a]					TSM$_{RUTL}$					TSM$_{PhUTL}$				
		RCP 4.5		RCP 8.5			RCP 4.5		RCP 8.5			RCP 4.5		RCP 8.5	
	current	2050	2070	2050	2070	current	2050	2070	2050	2070	current	2050	2070	2050	2070
1	10.7	12.2	12.5	12.9	13.8	3.6	2.1	1.8	1.4	0.5	6.29	5.55	5.25	4.85	3.95
2	11.2	12.7	12.9	13.4	14.2	3.1	1.6	1.4	0.9	0.1	5.79	5.05	4.85	4.35	3.55
3	13.9	15.3	15.6	16	16.8	0.4	−1	−1.3	−1.7	−2.5	3.09	2.45	2.15	1.75	0.95
4	13.6	15	15.3	15.8	16.6	0.7	−0.7	−1	−1.5	−2.3	3.39	2.75	2.45	1.95	1.15
5	13.9	15.3	15.6	16	16.8	0.4	−1	−1.3	−1.7	−2.5	3.09	2.45	2.15	1.75	0.95
6	10.2	11.7	12	12.4	13.2	4.1	2.6	2.3	1.9	1.1	6.79	6.05	5.75	5.35	4.55
7	11.4	12.8	13.1	13.5	14.3	2.9	1.5	1.2	0.8	0	5.59	4.95	4.65	4.25	3.45
8	11.4	12.8	13.1	13.5	14.3	2.9	1.5	1.2	0.8	0	5.59	4.95	4.65	4.25	3.45
9	11.4	12.8	13.1	13.5	14.3	2.9	1.5	1.2	0.8	0	5.59	4.95	4.65	4.25	3.45
10	11.4	12.8	13.1	13.5	14.3	2.9	1.5	1.2	0.8	0	5.59	4.95	4.65	4.25	3.45
11	12	13.5	13.7	14.2	15	2.3	0.8	0.6	0.1	−0.7	4.99	4.25	4.05	3.55	2.75
12	12.8	14.2	14.5	14.9	15.8	1.5	0.1	−0.2	−0.6	−1.5	4.19	3.55	3.25	2.85	1.95
13	10.7	12.1	12.4	12.8	13.7	3.6	2.2	1.9	1.5	0.6	6.29	5.65	5.35	4.95	4.05
14	13.4	14.8	15.1	15.6	16.4	0.9	−0.5	−0.8	−1.3	−2.1	3.59	2.95	2.65	2.15	1.35
15	14.3	15.7	16	16.4	17.2	0	−1.4	−1.7	−2.1	−2.9	2.69	2.05	1.75	1.35	0.55
16	13.7	15.2	15.5	15.9	16.7	0.6	−0.9	−1.2	−1.6	−2.4	3.29	2.55	2.25	1.85	1.05
Average						2.05				0.91	4.74				2.54

[a]Obtained from Worldclim database v. 1.4 (https://www.worldclim.org) at 30 arc-s resolution.
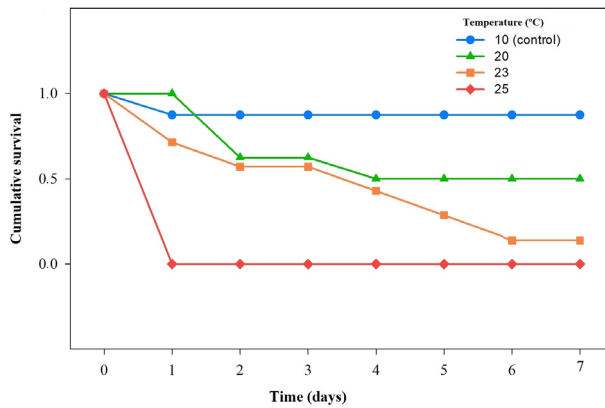
Fig 2. Kaplan–Meir survivorship curves for the four temperature treatments (indicated by colours) used to measure upper lethal limits. Each point represents survival probability (mean ± SE) at this treatment and time.

RCP 8.5) of a half of the caves is predicted to be higher than the RUTL, in none of them it would overcome the PhUTL (Table 2; Fig. 3c,d, Figs S2 and S3 for results considering other RCPs).

Discussion

Knowledge on organisms' thermal limits—the temperature range at which they can thrive—is essential for predicting survival under climate change. We found that the thermal limit of

our species obtained by physiological experiments is 3.3°C higher than that obtained from the distributional and climate data (i.e., the hottest cave in which the species occurs), meaning that the species could have some physiological capacity to deal with warming conditions *in situ*. If we focus on the thermal niche obtained from distributional and climate data, the risk of local extinctions would be high for at least a half of the populations of *N. vasconicum vasconicum*. However, this alarming message can be attenuated when considering physiological information. This in line with recent research on the modelling techniques to predict biodiversity changes under climate change, which shows that when ecophysiological traits (e.g., Kearney *et al.*, 2010; Benito Garzón *et al.*, 2019) and intraspecific variability (e.g., Zhang *et al.*, 2021) are taken into account, the resulting predictions are more realistic and less extreme than they would be by fitting a correlative SDM alone. Our results show that in the case of poor dispersal species, the estimates of species vulnerability to climate change are very sensitive to the approach used, and this sensitivity will depend on the extent to which the distribution of the species is limited by temperature (Pacifici *et al.*, 2015; Sánchez-Fernández *et al.*, 2016).

It is likely that the most realistic thermal limit of the studied species has a value between RUTL (14.3°C) and PhUTL (17.57°C). RUTL could underestimate thermal tolerance as the climatic conditions of the localities in which a species occurs could only represent a part of the full climatic conditions suitable for the species (Peterson *et al.*, 2011). Given the low dispersal capacity of subterranean species (Rizzo *et al.*, 2013, 2017), it is likely that *N. vasconicum vasconicum* cannot occupy much of
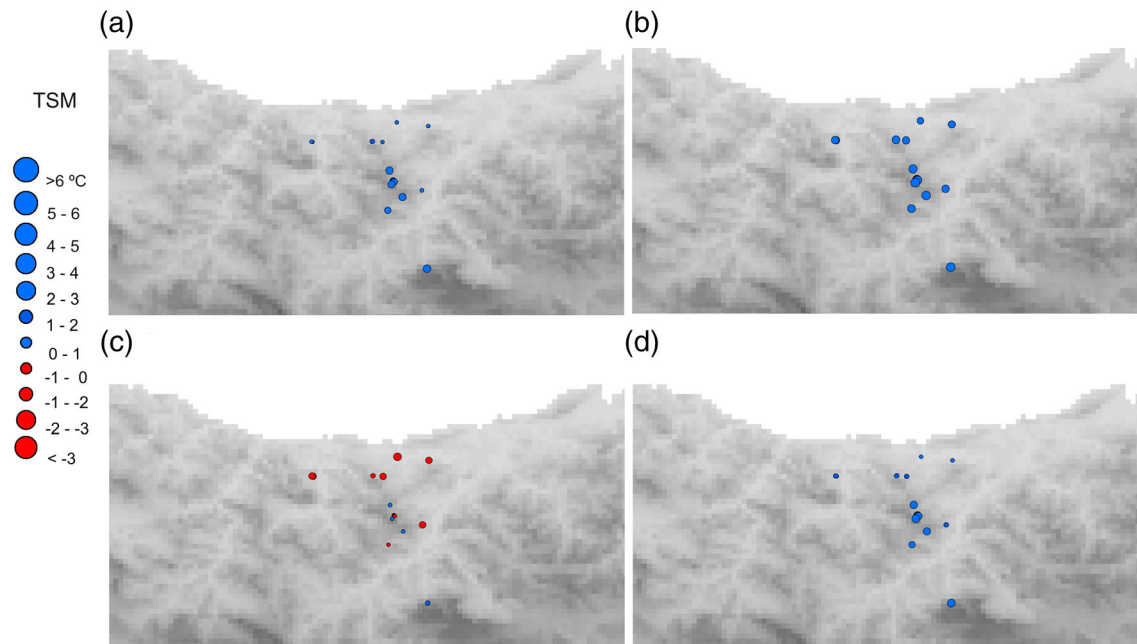
Fig 3. Thermal safety margins (TSM) for each locality, considering the thermal limits obtained from climate and distribution (realised upper thermal limits: TSM$_{RUTL}$) under current (a) and future (RCP 8.5; 2070) climatic conditions (c); and thermal limits obtained from physiological experiments (physiological upper thermal limits: TSM$_{PhUTL}$) under current (b) and future (RCP 8.5; 2070) climatic conditions (d). The greyscale of the background represents a gradient of altitude.

its thermally suitable habitat. Thus, physiological experiments on thermal tolerance could provide relevant information on this unoccupied climatic space.

The physiological thermal limit for our model species (PhUTL approximately 20°C) is similar to those obtained for other deep subterranean highly specialised species (e.g., Rizzo *et al*., 2015; Raschmanová *et al*., 2018; Pallarés *et al*., 2020), and lower than those found for other non-subterranean terrestrial pseudoescorpions (Anthony *et al*., 2016). A recent study shows that the specialisation process to live in deep subterranean habitats involves a loss of heat tolerance and this is irrespective of the temperature at which species are exposed (see Colado *et al*., unpubl. data). This lack of adjustment to local temperature could be interpreted as a result of the loss of costly regulatory mechanisms due to the extreme conditions of the deep subterranean environment (Rizzo *et al*., 2015). Thus, this thermal limit could be also similar for other Iberian subterranean pseudoscorpions, but further research would be needed to confirm such hypothesis.

Despite the valuable information provided by thermal tolerance experiments based on survival assays, such as the one conducted here, predictions of species vulnerability to climate change based on physiological data could be further refined by (i) considering the multiple factors that affect species thermal tolerance and (ii) focusing on thermally-sensitive traits others than survival. Regarding the first issue, subterranean habitats are at great advantage given by their extraordinary climatic stability. However, besides temperature, air moisture content (relative humidity) is an important limiting factor for terrestrial cave obligate species (Mammola *et al*., 2019b). Accordingly, the maintenance of high humidity levels appears to be essential for the survival of different troglobionts. This is generally explained by the high cuticular permeability of many species, associated with an extremely low resistance to desiccation (Howarth, 1980). In a context of changing rainfall patterns in many parts of the world, humidity levels may decrease in Mediterranean subterranean habitats, especially in the outer or more external parts, more connected to the surface environment (Xoplaki *et al*., 2004). But surprisingly, and as far as we known, there are no studies on the effects of such predicted decrease of relative humidity on the heat tolerance of subterranean species (nor in isolation nor in combination with the increase of temperature). Regarding the second issue, it is already well known that oxidative stress and alteration of key enzymatic activity could appear at temperatures below lethal limits (Pallarés *et al*., 2020). The difficulty to obtain laboratory cultures of most subterranean species and their long life cycles prevents the application of full life-cycle experiments (Castaño-Sánchez *et al*., 2020a), However, estimation of thermal limits in longer term exposures, in combination with other factors and from a metabolic/enzymatic perspective under sublethal conditions, could provide further ecological meaning of our previous findings, likely reducing the values of thermal limits obtained from survival experiments.

We used the UTL estimated here to identify other populations of our species that could be exposed to temperatures close to their lethal limits, assuming that inter-population variation in thermal tolerance would be minimal given the lack of phenotypic plasticity of such trait observed in deep subterranean species (Pallarés *et al*., 2021). Conservation recommendations related to climate change are mostly strategies based on the selection of protected areas and measures to increase habitat connectivity (Heller & Zavaleta, 2009; Arribas *et al*., 2012). However, for subterranean species, as happens to other species with poor dispersal capabilities, a more efficient and practical strategy must be to concentrate conservation efforts in actual localities (i.e., *in situ* management; Castaño-Sánchez *et al*., 2020a). Thus, we recommend to minimise anthropogenic pressures to avoid ecotoxicological effects (Castaño-Sánchez *et al*., 2020a) and monitor the populations from the caves with lowest TSMs, Altxerrikokoba and ZazpiIturri.

Shedding light on the relationship between thermal tolerance, subterranean specialisation and current climatic conditions of the habitat is pivotal for the conservation of subterranean fauna in a climate change perspective. With this study, we take advantage of the potential that the subterranean environment offers to develop the field of conservation physiology (Cooke *et al*., 2013) to provide a physiologically-based vulnerability assessment of an endemic species. However, much more effort is needed to (i) increase our knowledge on the thermal tolerance of subterranean fauna (Mammola *et al*., 2019a) and (ii) consider the neglected subterranean ecosystems in conservation and management policies. Complementary approaches to estimate thermal tolerance not only in subterranean species but also in other poor dispersal species could provide more accurate predictions of their capacity to face climate change.

Acknowledgements

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Survivorship and median lethal temperature (LT50) results at 7 days of exposure in the thermal tolerance experiment,

and log rank test (LRT) assessing the overall effect of temperature treatment on survival time.

Fig. **S1**. Areas with suitable temperature for *Neobisium vasconicum vasconicum* (in red) estimated from the climate of its known distribution for both current (A) and future climatic conditions (B: RCP 4.5 for 2050; C: RCP 4.5 for 2070; D: RCP 8.5 for 2050; E: RCP 8.5 for 2070). The areas with temperature below the minimum are represented in orange. The areas with temperature above the RUTL are represented in grey. Blue dots indicate current known current occurrences.

Fig. **S2**. Thermal Safety Margins for each locality, considering thermal limits obtained from climate and distribution under future climatic conditions (TSM_{RUTL}); (A: RCP 4.5, 2050; B: RCP 8.5, 2050; C: RCP 4.5, 2070; D: RCP 8.5, 2070). The greyscale of the background represents a gradient of altitude.

Fig. **S3**. Thermal Safety Margins for each locality, using thermal limits obtained from physiological experiments under future climatic conditions (TSM_{PhUTL}); (A: RCP 4.5, 2050; B: RCP 8.5, 2050; C: RCP 4.5, 2070; D: RCP 8.5, 2070). The greyscale of the background represents a gradient of altitude.

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