CONSEQUENCES OF THE GLOBAL CLIMATE CRISIS ON THE CAVE BEETLE *DARLINGTONEA KENTUCKENSIS* VALENTINE BASED ON THERMAL TOLERANCE AND DEHYDRATION RESISTANCE

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Abstract

Rising temperatures and diminishing groundwater availability due to the current climate crisis are predicted to expose cave faunas in eastern North America to unprecedented environmental conditions that could prove detrimental to their unique ecosystems. Organisms that inhabit relatively stable environments like caves are known to develop narrow physiological tolerances. Cave habitats with their organisms are simple ecosystems whose homogeneity offers an ideal system for testing the ability of a highly specialized fauna to tolerate abiotic changes. We tested the capability of a cave-specialized beetle in the eastern United States, *Darlingtonea kentuckensis* Valentine, to withstand future climatic shifts in its environment. We exposed individuals to a range of relative humidities and temperatures for 10 days. The data strongly suggest that there is a temperature threshold for the survival of *D. kentuckensis*, but it is a higher thermal tolerance than would be expected in an environment that has not fluctuated in recent evolutionary time and suggests remnant physiological characteristics of ancestral epigean carabids. Decreasing the relative humidity in the environment resulted in a much more dramatic decline in survival, indicating highly evolved specialization for constant high-humidity environments. The narrow humidity threshold in which troglobionts can survive may be a much more apparent limiting factor than temperature in adapting to climatic shifts within a cave environment.

INTRODUCTION

Caves in eastern Kentucky formed through the dissolution of limestone within deposits that began accumulating when shallow seas covered much of this area during the Mississippian Period that began about 360 Ma (Kentucky Geological Survey, 2021; Grabowski, 2001). Karst landscapes, in which water travels underground through carbonate rock rather than in above-ground streams, account for 20–25 % of all ice-free land surface on earth (Ford and Williams, 2007). Karst ecosystems provide homes to organisms that are highly specialized for the extreme conditions within these habitats (Barr, 1969; Culver et al., 2009; Romero, 2009).

It has been commonplace to deem caves as dismal and depauperate ecosystems due to their lack of primary productivity, and a relatively limited amount of ecological research has been done within these environments (Romero, 2009). In reality, some cave ecosystems are teeming with a surprisingly large array of taxa that have often convergently evolved various traits to survive in the generally low-energy, lightless environment they inhabit (Soares and Niemiller, 2020; Romero, 2009). For example, 18 different families of Coleoptera (beetles) have been reported to have colonized caves in different regions around the world (Romero, 2009). In North America, three families of this order, the Carabidae, Leiodidae, and Staphylinidae (Pselaphidae), include true obligate cave dwellers (troglobionts) (Barr, 1968; Peck, 1998).

The south-central Kentucky karst system is among the top 10 most endangered karst systems in the world (Romero, 2009), and the interior plateau is home to among the largest assemblages of obligate cave fauna in North America (Christman et al., 2005; Lewis and Lewis, 2005; Niemiller and Zigler, 2013). Further, eastern North American cave ecosystems are home to 170 described trechine cave beetle species with some endemic to single caves, as well as several critically endangered species (Barr, 1979; Christman et al., 2005). North American cave beetles are extremely diverse, with over 250 species estimated in the genus *Pseudanophthalmus* alone (Peck, 1998).

Eastern North American cave trechines were once thought to have evolved during glacial-interglacial transitions of the Pleistocene that concluded by warming and drying as the Holocene began (Barr and Holsinger, 1985), but cave beetles likely evolved much earlier than the Quaternary. If similar to the evolution of cave trechines in the Pyrenees (Faille et al., 2010), the time of origin may be around 10 Ma, an age that is close to an unpublished estimate of 13 Ma made by one of the authors (Philips) for the eastern US clade. In contrast, the cave trechines and a clade of subterranean European leiodids (Ribera et al., 2010) may have first appeared as early as the late Eocene about 35 Ma (Faille et al., 2013). Regardless of age of origin, fauna adapted for an earlier cooler, wetter surface climate are believed to have utilized caves originally as refugia but eventually became specialized troglobionts confined to life in caves (Barr and Holsinger, 1985). Long appendages, conversion to winglessness, and reduction or elimination of functional eyes are all

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unique troglomorphisms that hinder or even prevent cave-specialized organisms from dispersing outside of their cave refuges (Hedin, 1997; Snowman et al., 2010; Cardoso et al., 2011a; Cardoso et al., 2011b; Romero, 2011; Yao et al., 2016). There has been only one record of a troglobitic *Pseudanophthalmus* beetle occurring outside of a cave, but this was after heavy rains presumably washed the individual out from its cave habitat (Barr and Peck, 1965).

Cave environments have a constant temperature year-round that usually reflects the average annual temperature of the region but can fluctuate depending on elevation and presence of water (Tuttle and Stevenson, 2011). Caves that support subterranean life also exhibit relative humidities that do not reflect the conditions outside the cave and are typically much higher, as evaporation rates are near-zero within a short distance inside the cave entrance (Barr and Holsinger, 1985; Tuttle and Stevenson, 2011).

Inhabitants of relatively constant environments such as caves are known to exhibit narrower physiological tolerances (Futuyma and Moreno, 1988). For example, several specialized hypogean arthropods are limited to either a constant or narrow range of subterranean environmental conditions in which they are found (Delay, 1978; Huey and Kingsolver, 1989; Lencioni et al., 2010; Bernabò et al., 2011; Novak et al., 2014; Rizzo et al., 2015). Further, the effects of changing climates have already been noted in cave arthropods. Populations of highly specialized troglobitic spiders in the western Alps are declining, and it is suggested that this is due to slight temperature changes in their cave environments (Mammola et al., 2018). Other cave-specialized arthropods, like troglobitic and troglophilic beetles in the Alps, appear to exhibit tolerance to slight temperature increases for a short period of time but only up to a certain threshold (Rizzo et al., 2015; Pallarés et al., 2019).

Cave systems in Kentucky and nearby states normally average around 12–13 °C and nearly 100 % relative humidity (Marsh, 1969; Perry, 2013). The consistency of the conditions inside any cave is due to the insulation and protection offered by the cave substrate from weather changes happening above ground. The impact of constant temperature and humidity on the tolerance range of cave organisms has been little researched, but it is vitally important to understand how climate change and anthropogenic activity will impact cave faunas in the future. While the climate in caves will not change as quickly as in above-ground habitats, it is predicted to shift due to rising temperatures and changing precipitation patterns (Badino, 2004); indeed, the eastern United States is expected to become both warmer and wetter (US EPA, 2016). While rainfall is expected to increase in Kentucky, it will be countered by rising evaporation rates. One prediction suggests that groundwater recharge is expected to decrease 2.5–5 % each year, and droughts are likely to be increasingly severe (US EPA, 2016). While cave conditions are considered relatively constant, there is a direct association between the external climate and the cave environment (Moore and Nicholas, 1964; Smithson, 1991; Badino, 2004; Badino, 2010; Covington and Perne, 2015).

Climate change remains an existential threat to global biodiversity. As a part of the web of life, cave habitats and their species are simple ecosystems whose homogeneity offers an ideal model system for testing the ability of specialized fauna to tolerate abiotic changes within ecosystems (Rizzo et al., 2015). In that light, research was conducted on the cave-specialized beetle, *Darlingtonea kentuckensis* (Valentine, 1952). A robust population enabled one of the most statistically significant studies to date on the tolerance of a troglobiont to adverse (i.e., non-cave) conditions. Experiments were conducted by exposing individuals to both different relative humidities and temperatures to document their ability to survive. We hope this study improves our understanding of the ability of troglobionts to withstand future climate changes within their cave habitats.

MATERIALS AND METHODS

Target Species, Collection, and Holding Conditions

Darlingtonea kentuckensis Valentine is one of the better-known cave beetle species that has a large range in eastern Kentucky. A relatively large-bodied (7.3–7.7 mm) trechine, this beetle can have considerable populations with a hundred or more individuals present on a single sand bank in a cave. Here they hunt for their main food source of cave cricket (*Hadenoecus* sp.) eggs. This cave beetle acts as a top terrestrial predator in much of the Mississippian karst hypogean habitats located in a nearly 200-square-mile area on the eastern flank of the Cincinnati arch karst system (Boyd et al., 2020).

Adult specimens were collected by hand in July 2021 in Wind Cave, Pulaski County, Kentucky. The specific location may be obtained through the Kentucky State Nature Preserves Commission. This cave has a notably large population of *Darlingtonea*. Teneral individuals (freshly emerged adults with untanned exoskeletons) were excluded to maintain physiological consistency between individuals. Temperature at the site of collection was 11.8–12.2 °C, and it had a relative humidity (RH) of 95 % (measured with a HOBO Pro Series data logger).

Specimens were transported to Western Kentucky University (Bowling Green, Kentucky) in a Styrofoam cooler under controlled temperature and humidity to minimize the amount of stress on the beetles. A wet paper towel was placed in each transport container to maintain humidity (>90 % RH), and cooler packs were used to maintain a temperature of approximately 12 °C. An Onset HOBO Pro Series data logger placed in the transport container confirmed

cave conditions were maintained during transport. Once in the lab, specimens were placed in plastic containers (15 cm × 15 cm) with a moist plaster of paris (DAP) layer (approximately 1 cm thick) and acclimatized in a humidity control chamber at 12 °C (Intellus environmental controller chamber, Percival Scientific) for two days prior to placement into different conditions. To avoid starvation stress, frozen *Drosophila* were provided *ad libitum* to each container during acclimatization and replenished throughout the experiment.

Dehydration Resistance

To estimate the tolerance to desiccation, survival was assessed at different relative humidities for seven days. Four Pyrex knob-top nonvacuum glass desiccators (approximately 30 cm × 30 cm, from Fisher Scientific) were placed inside a Percival Scientific Intellus environmental control chamber and the temperature was kept at constant cave temperature (12 °C) for all four treatments to avoid any thermal stress. Ratios of glycerol/water mixtures in the bottom of each humidity control chamber were used to achieve 50 %, 65 %, 80 %, and 100 % RH and were confirmed by Onset HOBO Pro Series data loggers. The chambers were allowed to stabilize for two days before specimens were introduced. A high-walled glass dish of specimens (n = 10) was placed on a shelf above the glycerol/water mixture within each chamber. Survival was checked every 12 hours, and beetles were recorded as dead (no movement when agitated with a brush) or alive (any kind of movement including when agitated with a brush).

Basal Heat Tolerance

Survival rates at different temperatures were used to estimate heat tolerance. Containers of beetles (n = 18–20) were placed into five treatments for 10 days at 12 °C, 16 °C, 20 °C, 24 °C, and 30 °C using Intellus environmental control chambers (12 °C and 20 °C) and VWR incubators (16 °C, 24 °C, and 30 °C). High humidity (>95 % RH) was maintained throughout the experiment by wetting paper towels within the container to avoid any desiccation stress. Survival was checked every 12 hours and was recorded as dead or alive as in the previous experiment.
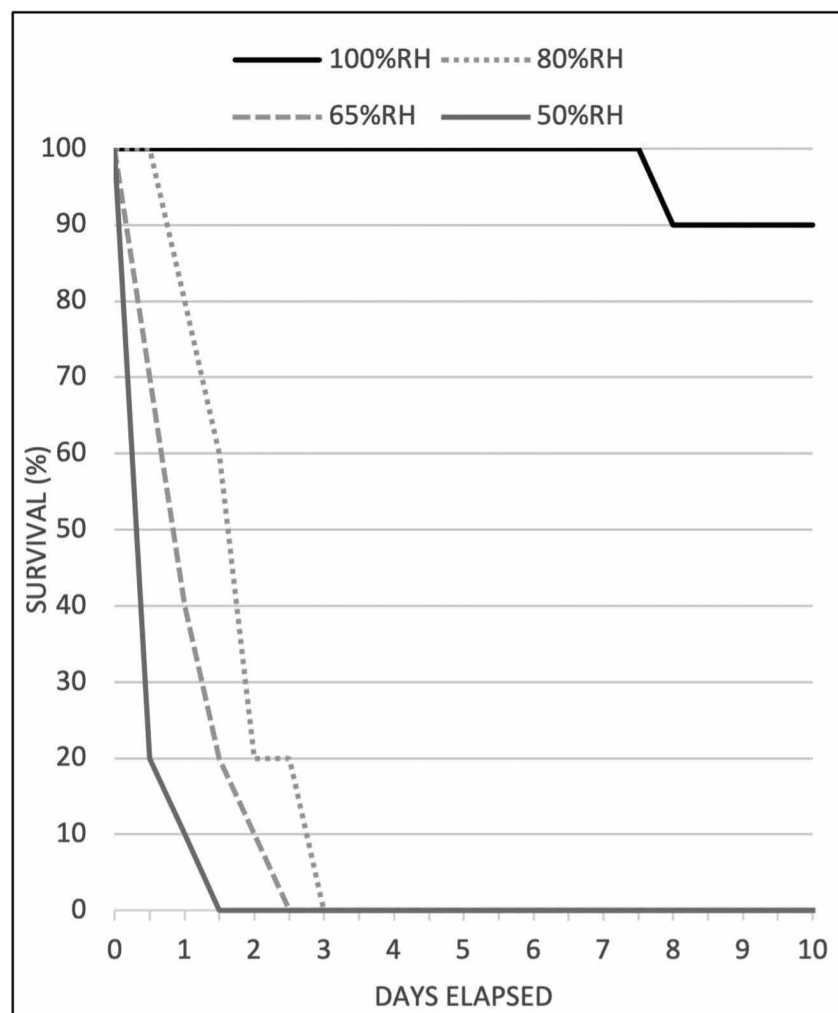
Data Analysis

Non-parametric Kruskal-Wallis and Mann-Whitney U-tests were used to evaluate the difference in survival at various temperatures and humidities. These tests are ideal for testing if there is a significant difference between two or more groups of an independent variable with ordinal values (i.e., the different temperatures and humidities) that were used in this study. Multiple comparison tests were also conducted to detect significant differences between treatments. All statistical analysis was conducted using VASSARSTATS (vassarstats.net).

RESULTS

Dehydration Resistance

Beetles exposed to conditions drier than that of a cave environment (50 %, 65 %, and 80 % RH) did not survive longer than three days once in treatment (Fig. 1). The closer the humidity was to 100 %, the longer beetles survived (Fig. 2). A Kruskal-Wallis test indicated that the survival of *D. kentuckensis* differed significantly between normal cave conditions and the drier artificial conditions (H(3) = 29.7, p <0.0001; Table 1). Additional pairwise Mann-Whitney *U*-tests indicated that survival in all 3 drier treatments differed significantly from the natural cave environment and from each other (Table 2).

Figure 1. Percentage survival throughout the experiment at respective relative humidities.
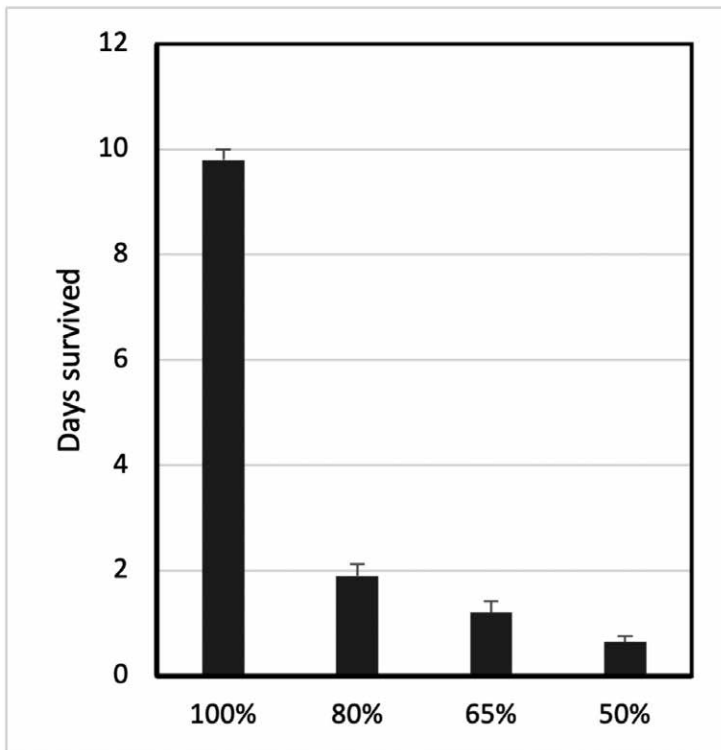
Figure 2. Average number of days *D. kentuckensis* survived at respective relative humidities. Error bars indicate standard error (*n* = 10).
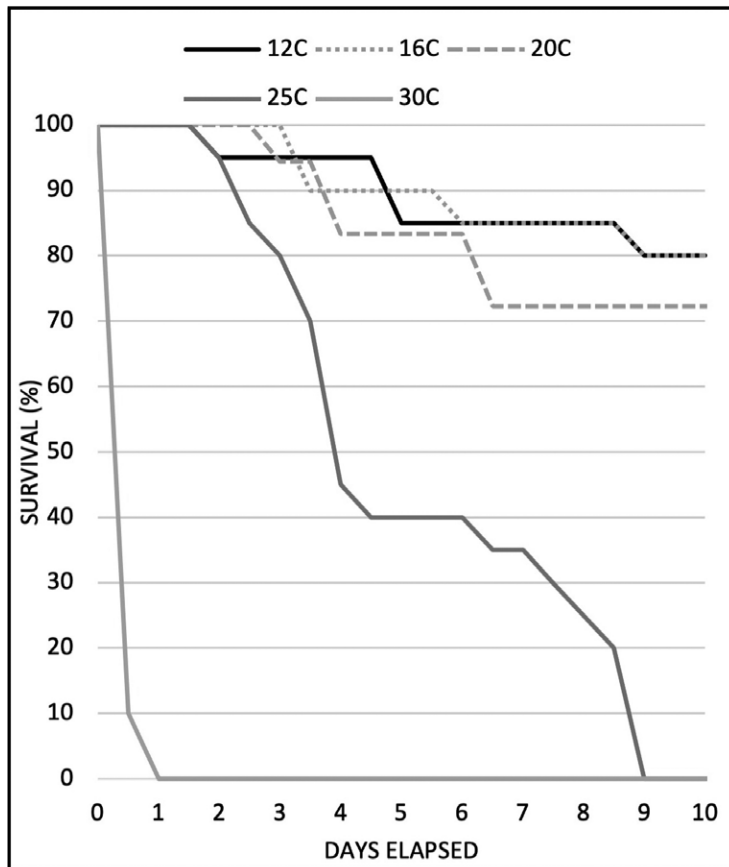

Figure 3. Percentage survival throughout the experiment at respective temperatures.

Basal Thermal Tolerance

Sample populations of *D. kentuckensis* were tolerant to higher temperatures for relatively long periods of time (Fig. 3), although beetles survived for less time when exposed to the highest temperatures (Fig. 4). A Kruskal-Wallis test indicated that there was a significant difference in *D. kentuckensis* survival when exposed to conditions warmer than their cave environment (H(4) = 64.66, *p* <0.0001; Table 1).

Interestingly, the 12 °C, 16 °C, and 20 °C data did not differ significantly from one another when analyzed with pairwise Mann-Whitney *U*-tests. At the highest temperature of 30 °C, beetles did not survive more than 24 hours and their average survival time was significantly less than all of the other four temperatures tested (*p* <0.0001, *n* = 20). At the next-highest temperature of 24 °C, the population (*n* = 20) survived for significantly less time than cave conditions (*p* <0.0001), but survival was significantly higher than at 30 °C (*p* <0.0001). The next two lowest temperatures, 20 °C and 16 °C, did not show any significant variation from survival under cave conditions (Table 3).

DISCUSSION

The physiological mechanisms behind tolerance to a great range of conditions are processes that are energetically costly and could have been selected against once confined to a stable environment (Krebs and Loeschcke, 1994; Monoghan et al., 2009; Tomanek, 2008). It is thought that the troglophilic, ancestral fauna adapted to cooler, wetter conditions took refuge in caves, sinkholes, ravines, leaf litter, and deep soil to avoid desiccation when the surface environment dried and warmed (Barr, 1968; Boyd et al., 2020). Prolonged orthogenesis under relatively stable conditions in caves (and possibly in deep soil) resulted in the eventual shift from a troglophilic to a troglobitic species (Barr, 1968).

Thermal Tolerance

The climatic variability hypothesis (Stevens, 1989) states that species from more thermally stable environments should have much narrower thermal tolerance breadths and reduced acclimation capacity than species from more climatically variable environments. This higher sensitivity to thermal variability has been observed in both invertebrates and vertebrates found in environments that maintain stable temperatures (Di Lorenzo and Galassi, 2017; Feder, 1978; Somero, 2005; Tomanek, 2008; Shah et al., 2017; Markle and Kozak, 2018). In contrast, tolerance to a great range of temperatures in some species inhabiting stable environments have been observed, such as in troglobitic leiodid beetles in Europe that have maintained a high level of thermal plasticity (Pallarés et al., 2020).
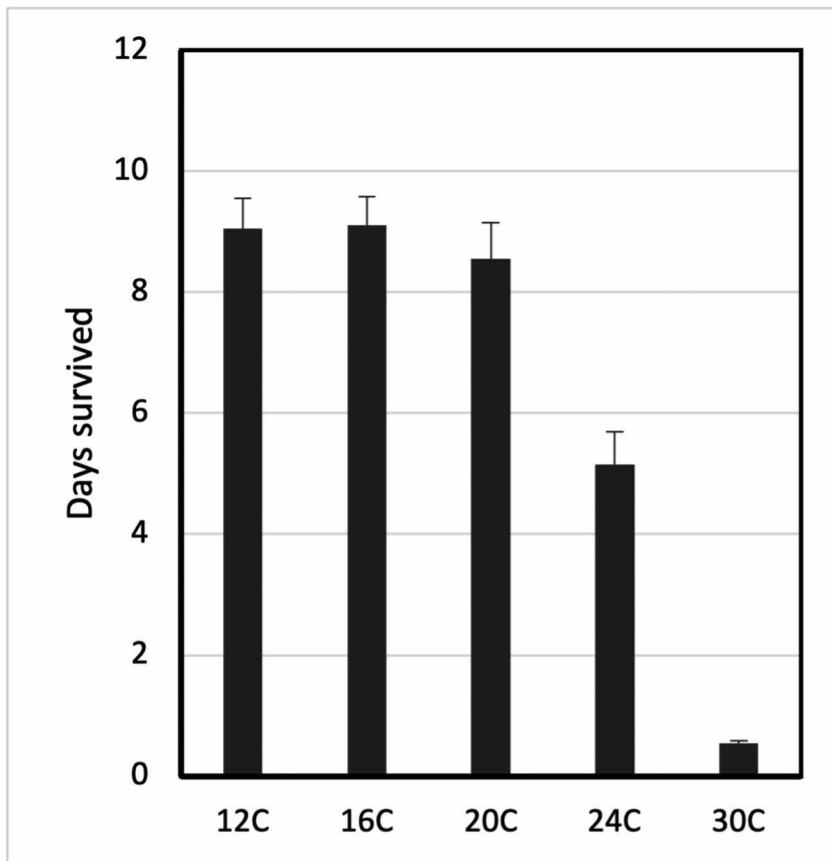
Figure 4. Average number of days *D. kentuckensis* survived in different temperatures. Error bars indicate standard error (*n* = 18–20).

The thermal tolerance of *D. kentuckensis* was surprisingly broad. Beetle survival only began to decline significantly between 20 °C and 24 °C (Figs. 3 and 4), which is 7–11 °C higher than any temperature they or their ancestors presumably would have had to endure for several million years or more. The data suggest there is a temperature threshold of slightly higher than 20 °C for the survival of *D. kentuckensis*. Troglobitic and troglophilic coleopterans have exhibited similar temperature thresholds (20–25 °C) in cave systems around the world (Pallarés et al., 2019; Rizzo et al., 2015).

While the fauna found in thermally stable environments is expected to exhibit a relatively narrow thermal tolerance, this may be shaped by its evolutionary history. Lineages of troglobitic fauna that are believed to have colonized caves earlier in their evolutionary history are thought to correspond with higher thermal sensitivity. In contrast, fauna that has colonized caves more recently may still exhibit greater tolerances remnant of its phylogenetically closer generalist epigean ancestors (Pallarés et al., 2019). As the climate of eastern North America was highly variable during glacial-interglacial transitions and much less so via annual seasonal fluctuation, troglophilic ancestors may have had wide temperature tolerance breadths which could still exist in their extant troglobitic descendants despite their now-stable environment

Table 1. Results of Kruskal-Wallis *K*-tests to evaluate whether the population medians on temperature and relative humidity are statistically different across all levels.

Environmental Factor	Kruskal-Wallis *K*	d.f.	*p*-value
Temperature	64.66	4	<0.0001*
Relative Humidity	29.7	3	<0.0001*

Table 2. Respective *p*-values, U values, and *z*-values for pairwise Mann-Whitney U-tests comparing survival at different relative humidities (*n* = 10).

Treatment Comparison, %	*z*-value	U-value	*p*-value
100 vs 80	3.74	0	<0.0001*
100 vs 65	3.74	0	<0.0001*
100 vs. 50	3.74	0	<0.0001*
80 vs. 65	2.0	23	0.0228*
80 vs. 50	3.44	4	0.0003*
65 vs. 50	1.97	23.5	0.0244*

(Issartel et al., 2005; Mermillod-Blondin et al., 2013; Rizzo et al., 2015; Pallarés et al., 2019).

There were observable behavioral differences in populations with varied temperatures. At 12 °C, the beetles were notably active but did not seem to eat or tamper with

Table 3. Respective *p*-values, U-values, and *z*-values for pairwise Mann-Whitney U-tests comparing survival at different temperatures (*n* = 18−20).

Treatment Comparison, °C	*z*-value	U-value	*p*-value
12 vs. 16	0	200.5	0.50
12 vs, 20	−0.42	195	0.337
12 vs. 24	4.45	35	<0.0001*
12 vs. 30	5.40	0	<0.0001*
16 vs. 20	−0.39	194	0.3483
16 vs. 24	4.36	38.5	<0.0001*
16 vs. 30	5.40	0	<0.0001*
20 vs. 24	3.74	51.5	0.0001*
20 vs. 30	5.25	0	<0.0001*
24 vs. 30	5.40	0	<0.0001*

any dead beetles. In contrast, beetles in the 16 °C treatment were observed carrying pieces of beetle carcasses in their mandibles and likely scavenging, although predation cannot be rejected. No disarticulation of carcasses was observed in the three higher temperature regimes, but activity had notably increased. Large cave trechines have very low metabolisms that allow them to sustain themselves for weeks on a single meal (Griffith and Poulson, 1993). A single egg can satiate an adult *Neaphaenops* (a taxon native to central Kentucky and morphologically similar to the study species) for more than two weeks in normal cave conditions (Smith, 1986). It is possible that sustained exposure at a higher temperature caused a rise in metabolism, resulting in a strong need to replenish energy stores via feeding. This shift in metabolism could significantly impact long-term survival in *D. kentuckensis* and other troglobionts that have highly specialized physiologies adapted to a decreased, erratic food supply in most caves (Barr, 1968).

Dehydration Resistance

The results from exposing populations of *D. kentuckensis* to lower-than-normal relative humidities were dramatic and suggest highly evolved specialization for high humidity environments. With temperature at a constant 12 °C (as in their cave habitat), no beetles in any treatment outside of the 100 % RH control (i.e., 80 %, 65 %, and 50 % RHs) survived longer than three days (Fig. 1). There were significant differences in survival across all four relative humidities, with each having a significantly lower average survival than the next highest humidity (Fig. 2). The narrow humidity threshold in which troglobionts can survive is apparently a much more limiting factor than temperature in adapting to future abiotic shifts within their cave habitat.

Preference for high humidities is not uncommon in epigean beetle species (Neve, 1994; Šustek et al., 2017; Kirichenko-Babko et al., 2020), and only a physiological comparison of *D. kentuckensis* and an epigean sister species could reveal if the lack of desiccation resistance is a cave adaptation and not an ancestral trait shared by other trechines. According to Maddison et al. (2019), an epigean trechine beetle closely related to *D. kentuckensis* is the genus *Trechus* Clairville. Physiology research conducted on any epigean relatives of *D. kentuckensis* is lacking, but ecological studies indicate that at least one *Trechus* species (*T. quadristriatus* Schrank) has a preference for relatively dry conditions that may indicate a high tolerance for low humidities (Kriegel et al., 2021).

The limited dehydration resistance observed in *D. kentuckensis* is consistent with previous studies focusing on troglobitic desiccation tolerance. Cave cricket species in the eastern United States with increased troglomorphic adaptation have much lower desiccation tolerances than species closely related but less troglomorphic (Yoder et al., 2011). Troglobitic carabid beetles in Australia are completely absent in caves with low or varying relative humidity, whereas caves with adequate moisture hold abundant populations (Humphries and 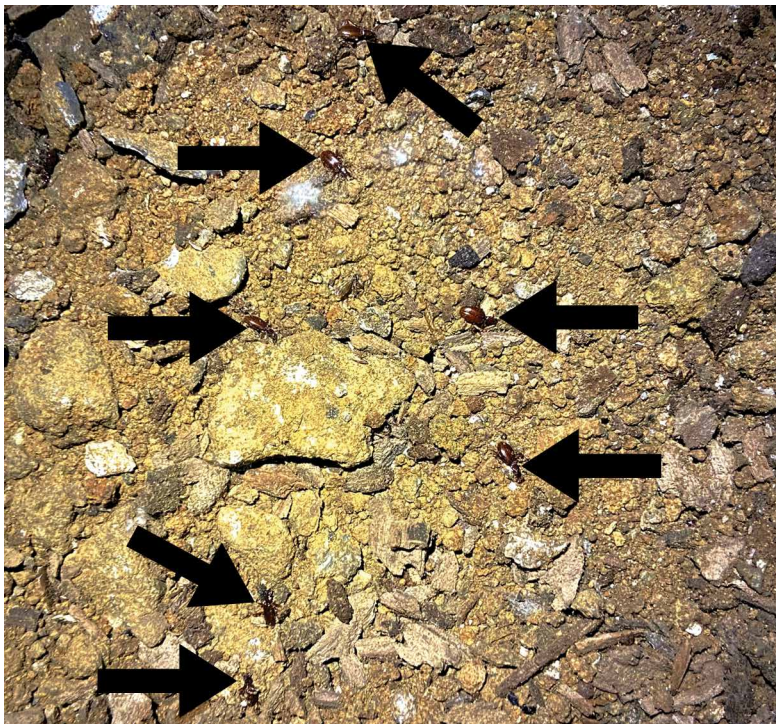Collis, 1990). Even in vertebrates, the cave adapted coqui frog species, *Eleutherodactylus cooki* Grant, demonstrates higher rates of water loss than its close epigean relative *E. coqui* Thomas (Rogowitz et al., 1999). Lastly, a pattern of high humidity dependency by *D. kentuckensis* may have been observed by the authors in Climax Cave (Rockcastle County, Kentucky) in the eastern United States; an apparent "death zone" was discovered with noticeably drier conditions after climbing up to an elevated region deep in the cave. Here, numerous remains of beetles were discovered, and no live individuals were found (Fig. 5).

The wider-than-expected thermal tolerance breadth observed in *D. kentuckensis* suggests remnant physiological characteristics of ancestral troglophilic carabids. In contrast, the narrow humidity tolerance suggests that a decrease in epigean habitat moisture in the past could better explain the eventual restriction of now-troglobionts to a subterranean existence. In summary, a wide range of thermal tolerance may have been necessary for survival during the transition to cave life, while the necessary humid conditions could be found through microhabitat refugia.

Figure 5. Apparent "death zone" discovered in Climax Cave, Rockcastle Co., Kentucky, where conditions were notably drier than areas of the cave containing live individuals. Arrows indicate remains of dead *D. kentuckensis*.

Climate Change Implications

With obvious sensitivity to humidity differences in its environment, *D. kentuckensis* and presumably other troglobionts with similar evolutionary histories are under serious threat from altered conditions in their subterranean environment. Theoretical models (Badino, 2004; Covington and Perne, 2015) and direct field observations (Domínguez-Villar et al., 2015) suggest that the underground climate may be significantly influenced by anthropogenic climate change. The United States Environmental Protection Agency estimates that groundwater recharge in Kentucky is decreasing 2.5−5 % annually. Droughts in Kentucky are also expected to become increasingly frequent and severe from altered precipitation patterns due to climate change (US EPA, 2016).

Notably, the effect of epigean climate change on the hypogean environment is not immediate; there is typically a time lag of up to several decades underground due to the thermal inertia properties of caves (Domínguez-Villar et al., 2015; Badino, 2004). Cascade effects on both physical and biological components of cave ecosystems are likely due to the accumulation of energy fluxes from the atmosphere to the underground. For example, the effect on air density gradients will modify air circulation in caves through small temperature changes, causing potential fallout in condensation and erosion processes, speleothem growth, and seasonal ventilation rates (Domínguez-Villar et al., 2015).

The stability of cave temperature is largely due to the insulation provided by the carbonate rock layer, which is at the thermal equilibrium of the water running through it from precipitation. As climate change increases temperatures above ground, the warmer water flowing through the karst will eventually cause a rise in cave temperature (Badino, 2010). Although a concern, this may not be dramatic enough to cause population declines in *D. kentuckensis* compared to the effect of lower humidity.

We predict that changes in temperature, groundwater availability, and humidity will expose cave fauna to unprecedented novel environmental conditions. Changes in the physical properties of the cave air (both temperature and humidity) are understood to be the most important aspects that humans will impact within these unique terrestrial ecosystems. In addition to climate change, agriculture, oil and gas extraction, expanding transportation corridors, and urban development are all leading threats to the south central Kentucky Karst ecosystem (Romero, 2009).

Behavioral thermoregulation through microhabitat selection is reduced in troglobionts due to their restriction to cave habitats. Further issues for species endemic to caves and other isolated habitat fragments such as islands and mountain summits are limited distributions, low recolonization potential, and reduced vagility that all hinder dispersal to new habitat (Hedin, 1997; Snowman et al., 2010; Yao et al., 2016; Cardoso et al., 2011a; Cardoso et al., 2011b). Based on this research, the climate crisis with its current trajectory could result in the irreversible failure of hypogean ecosystems including high levels of troglobiont extinction.

SUMMARY

The results from exposing populations of *D. kentuckensis* to different relative humidities suggest highly evolved specialization for high humidity environments. The narrow humidity threshold in which these beetles can survive is a much more apparent limiting factor than increasing temperatures in adapting to climatic shifts within a cave environment. The wider-than-expected thermal tolerance in *D. kentuckensis* suggests remnant physiological characteristics of ancestral troglophilic carabids. Changes expected in temperature, groundwater availability, and humidity within caves are predicted to expose this fauna to unprecedented novel environmental conditions as the climate crisis continues to pose an existential threat to this small but unique part of global biodiversity.

ACKNOWLEDGMENTS

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