

Some like it hot: Thermal preference of the groundwater amphipod *Niphargus longicaudatus* (Costa, 1851) and climate change implications

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ABSTRACT

Groundwater is a crucial resource for humans and the environment, but its global human demand currently exceeds available volumes by 3.5 times. Climate change is expected to exacerbate this situation by increasing the frequency of droughts along with human impacts on groundwater ecosystems. Despite prior research on the quantitative effects of climate change on groundwater, the direct impacts on groundwater biodiversity, especially obligate groundwater species, remain largely unexplored. Therefore, investigating the potential impacts of climate change, including groundwater temperature changes, is crucial for the survival of obligate groundwater species. This study aimed to determine the thermal niche breadth of the crustacean amphipod species *Niphargus longicaudatus* by using the chronic method. We found that *N. longicaudatus* has a wide thermal niche with a natural performance range of 7–9 °C, which corresponds to the thermal regime this species experiences within its distribution range in Italy. The observed range of preferred temperature (PT) was different from the mean annual temperature of the sites from which the species has been collected, challenging the idea that groundwater species are only adapted to narrow temperature ranges. Considering the significant threats of climate change to groundwater ecosystems, these findings provide crucial information for the conservation of obligate groundwater species, suggesting that some of them may be more resilient to temperature changes than previously thought. Understanding the fundamental thermal niche of these species can inform conservation efforts and management strategies to protect groundwater ecosystems and their communities.

CRedit author statement

Mattia Di Cicco: Conceptualization, Methodology, Data curation, Software. Tiziana Di Lorenzo: Methodology, Resources, Writing, Conceptualization. Barbara Fiasca: Term, Validation, Supervision Emma Galmarini: Methodology, Writing. Iliaria Vaccarelli: Methodology, Writing. Francesco Cerasoli: Validation, Software, Writing. Agostina Tabilio Di Camillo: Methodology, Writing. Diana Maria Paola Galassi: Conceptualization, Supervision, Funding acquisition, Writing, Validation

1. Introduction

Groundwater, which accounts for nearly all (96.3%) of the liquid freshwater immediately available for human activities and ecological processes, is in high demand globally, with human request estimated to exceed available volumes by 3.5 times the actual groundwater natural renewal capacity (Gleeson et al., 2012). Climate change is expected to worsen this scenario by increasing the frequency of droughts and human impacts on groundwater and the ecosystems that depend on it (Stigter et al., 2023). Although some studies have investigated the quantitative

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effects of climate change on groundwater in terms of changes in hydraulic regime, saline intrusion, water temperature, and oxygen concentration (Kløve et al., 2014 and references therein; Rouillard et al., 2021), the direct impacts of climate change on groundwater ecosystems have been still insufficiently studied or simply inferred (Mammola et al., 2019). Groundwater ecosystems host specialized organisms that can survive and thrive in habitats that are typically energy- and oxygen-limited yet thermally stable (Malard et al., 2023a,b). Groundwater obligate species (i.e., species that complete their life cycle in groundwater and show adaptations to the subterranean environment) contribute to global diversity and provide valuable ecosystem services, including organic carbon processing and nutrient recycling, depending on their feeding and non-trophic engineering activities (Boulton et al., 2008, 2016; Deharveng et al., 2009; Tuinstra and van Wensem, 2014; Griebler and Avramov, 2015; Sánchez-Fernández et al., 2021). Given that climate change poses a significant threat to groundwater (Holman, 2006; Bovolet al., 2009; Gunawardhana and Kazama, 2011; Benz et al., 2017; Hemmerle and Bayer, 2020; Wunsch et al., 2022), understanding the potential impacts of temperature increase on groundwater communities is critical to predicting the cascade effects on the ecosystem services they provide.

An effective approach to predict the effects of climate change on a species is to evaluate its thermal niche (Gvoždík, 2018), particularly its thermal niche breadth, i.e., the difference between the upper and the lower thermal range limits. The thermal niche constitutes one of the n-dimensions of the ecological niche, which was defined by Hutchinson (1957) as a conceptual “hypervolume” delimited by the ranges of several environmental conditions (temperature included) in which an organism can survive and reproduce (Pörtner, 2010; Grigaltchik et al., 2012; Angilletta et al., 2019). Because groundwater is a thermally stable environment, scientists have long hypothesized that organisms that are dependent on it have a small thermal niche breadth. Even if evidence supporting this theory is growing (e.g., Issartel et al., 2005; Colson-Proch et al., 2009; Mermillod-Blondin et al., 2013; Galassi and Di Lorenzo, 2017; Jones et al., 2021), a conclusive finding is still a long way off due to the paucity of studies and their not-always-coherent results (Di Lorenzo et al., 2023a). For instance, Mermillod-Blondin et al. (2013) found that three groundwater obligate species of the isopod *Proasellus* have different tolerances to temperature increases: while two of these species are sensitive to changes in temperature (2 °C below and above their habitat temperature), the third species, *Proasellus valdensis* (Chappuis, 1927), exhibits a higher thermal tolerance breadth (11 °C). On the other hand, Di Lorenzo and Reboleira (2022) found that *Proasellus lusitanicus* (Frade, 1938), an endemic species of the Estremanho Massif in Portugal, exhibits narrow thermal plasticity, being unable to acclimate to temperatures exceeding +1.5 °C that of the collection site, as also observed for *Moraria* sp., a copepod species endemic to the Corchia Cave in Italy (Di Lorenzo et al., 2023b). Different thermal niche breadths translate into different species’ distribution ranges (Eme et al., 2014; Esmaili-Rineh et al., 2019). So far, a narrow thermal niche seems to be typical of groundwater obligate species that have a restricted, almost point-like, distribution. These species would be likely unable to adapt to a scenario of future climatic change. However, the risk scenario for widely distributed groundwater species is not readily apparent.

Groundwater amphipods are a diverse, widely-distributed, and ecologically important group of freshwater invertebrates. They play a crucial role in aquatic ecosystems by processing detritus and maintaining sediment hydraulic connectivity (Pinchuk et al., 2013; Stumpp and Hose, 2017). They are commonly used in biodiversity monitoring and ecotoxicology tests (Altermatt et al., 2014; dos Santos et al., 2022). The genus *Niphargus* Schiödte, 1849, which is the most species-rich taxon of obligate groundwater dweller amphipods in the world with over 430 species described (Fiser, 2019), is distributed in the Western Palearctic (Zagmajster et al., 2014; Esmaili-Rineh et al., 2019). *Niphargus* species show troglomorphic traits (i.e., they are depigmented, eyeless and have a long life span and low metabolism; Venarsky et al., 2023) and are

known from all types of groundwater habitats, including the unsaturated and saturated karst, alluvial aquifers and hypohelminthic habitats, as well as from ecotones such as groundwater-fed springs, and hyporheic zones (Borko et al., 2021). A few species successfully recolonized the ecotone between surface and subterranean ecosystems, and thrive in surface water bodies, where they seek refuge in some microhabitats mirroring the subterranean environments (Copilaş-Ciocianu et al., 2018; Fišer, 2019). Finally, some *Niphargus* species that live in ecotonal habitats like springs appear to exhibit a circadian rhythm, being much more active at night (when there is a lower risk of predation and when they explore habitats on the surface that are richer in food) than during the day (when they seek protection underground from predators). This suggests that some *Niphargus* species can withstand thermal variations of several degrees between day and night (Manenti and Barzaghi, 2021).

Empirically estimating the thermal niche of long-living organisms poses challenges. While direct estimation methods exist, they are limited to species characterized by short-generation times, such as bacteria, nematodes, and fruit flies (Siddiqui and Barlow, 1972; Cooper et al., 2001; Anderson et al., 2011). To address this limitation, the “chronic method” or “optimality approach” may provide indirect but robust means of estimating the thermal niche (Reynolds and Casterlin, 1979; Gvoždík, 2018). This approach entails exposing specimens to a thermal gradient for multiple days, devoid of any biotic or abiotic restrictions. It assumes that, in unconstrained conditions, organisms will choose the thermal range that optimizes their reproductive fitness. The chronic method described here is a simple way of estimating the thermal niche in its narrow sense; however, it has not been intended as an alternative approach to determine the critical temperature limits (CT_{max}).

This study aims to delimit the fundamental thermal niche (i.e., thermal niche in the absence of interspecific interactions) of the groundwater obligate amphipod species *Niphargus longicaudatus* (Costa, 1851) by determining its preferred temperature range through the “chronic method” by exposing individuals to a horizontal controlled temperature gradient for six days. In addition, the study examines the temporal dynamics of the results, including an analysis of whether the animals exhibit a shift in their overall thermal preference along the gradient from the beginning to the end of the trial. Our hypothesis was that the thermal niche of *N. longicaudatus* would span several degrees centigrade, in accordance with its relatively wide geographic distribution.

2. Materials and methods

2.1. 1 amphipod collection, handling and acclimation

Niphargus longicaudatus is a complex of groundwater obligate amphipod cryptic species with one of the broadest geographic ranges among the genus, being found in Corsica, Italy, the eastern Adriatic coast and Greece (Barnard and Barnard, 1983), its geographic range spanning over 1500 km (Lefébure et al., 2007; Borko et al., 2023). We sampled sixty adult individuals of *N. longicaudatus* (length: 10.21 ± 1.33 mm; width: 2.28 ± 0.33 mm; Fig. 1a) in Stiffe Cave, Central Italy (42°15'20.62"N 13°32'32.51"E; altitude 696 m a.s.l.; Fig. 1b) from September to November 2022. Specimens were collected with hand-made bottle traps and transported within an hour to the laboratory (University of L'Aquila, Central Italy) in a 1 L plastic container filled with cave water. In the laboratory, sampled individuals were sorted and stored in an aquarium containing 2 L of 0.2 µm-filtered cave water (culture medium; major ions concentrations: SO₄²⁻ = 9.60 ± 1.21 mg/L; Cl⁻ = 10.40 ± 2.58 mg/L; K⁺ = 2.9 ± 0.20 mg/L; Ca²⁺ = 93.64 ± 11.79 mg/L; Na⁺ = 7.79 ± 1.85 mg/L; electrical conductivity = 476.71 ± 14.84 µS cm⁻¹) to provide a minimum viable volume of 2 mL for each individual (Di Lorenzo et al., 2019). The aquarium was kept in a thermostatic cabinet (Velp Scientifica™ FOC 120 E Cooled Incubator) at 9 ± 0.2 °C (mean annual temperature at the sampling site; Di Cicco et al., 2021), in permanent darkness till the start of the experiment in February

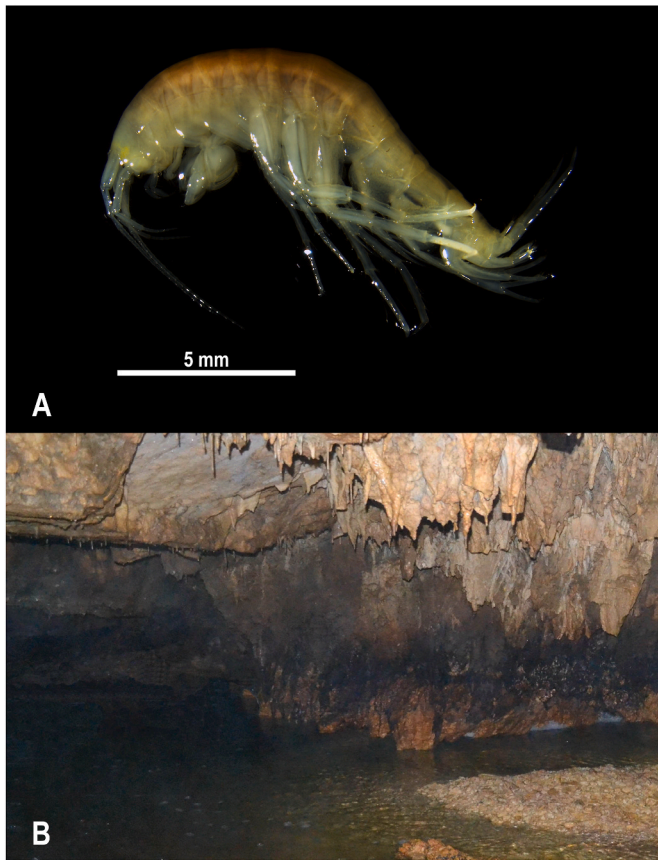


Fig. 1. A - An adult individual of *Niphargus longicaudatus* (Costa, 1851) sampled from Stiffe Cave (Abruzzo, Central Italy); B - *N. longicaudatus* sampling site inside Stiffe Cave.

2023. Culture medium was renewed once a week, and the individuals were fed on a commercial food (20 g L^{-1} ; Vipargan, Sera, Germany).

2.2. Description of the horizontal thermogradient apparatus

Temperature preference was determined by placing the animals into a rectangular metal tank (height = 5 cm, width = 5 cm, length = 110 cm) filled with 0.5 L of $2 \mu\text{m}$ -filtered cave water (Reynolds and Casterlin, 1979; Golovanov, 2013; Verbitsky et al., 2017, 2018). To generate the thermal gradient (from $7.0 \pm 1.0 \text{ }^\circ\text{C}$ to $22.0 \pm 1.0 \text{ }^\circ\text{C}$), we placed, at the opposite sides of the tank, two tubular coils (radius = 2.5 cm) in which cold and hot water was pumped in by two temperature-controlled circulating water baths (CB 5–10, ARGOLAB®, Italy). Each coil was submerged in water. For temperature readings, we placed 11 digital thermometer probes (precision = $\pm 0.1 \text{ }^\circ\text{C}$) at equidistant locations along the tank (Fig. 2). The external sides of the tank were covered with 1 cm-thick polystyrene layer to reduce heat exchange with the

environment. On the top of the tank, we placed a 2 mm-thick dark metal panel that we covered with polystyrene blocks to: i) avoid heat exchange and minimize water loss through evaporation, ii) maintain darkness inside the tank between different animal counting phases. A stable and linear horizontal thermal gradient was achieved 48 h after the start-up of the circulating water baths (for details about the linearity check, see section 2.4). Prior to the experiment, preliminary tests were carried out to assess potential cannibalistic or aggressive behavior. In detail, we put 30 adult individuals in another tank with microfiltered sampled cave water for 7 days as a control (mean temperature = $9.0 \text{ }^\circ\text{C}$). At the end of the trial, we observed that the animals actively explored the entirety of the tank yet did not display any remarkable instances of intraspecific interactions. Specifically, we did not observe clusters of individuals in specific areas of the tank, nor did we find any injured or dead individuals. Therefore, we decided to use the same timing for the gradient experiment of 24 h of acclimation followed by six days of observation.

2.3. Experimental procedure

Thirty adult individuals of *N. longicaudatus* were gently placed, by using a soft brush, inside the tank in the zone corresponding to the acclimation temperature ($9.0 \text{ }^\circ\text{C}$). Animal observation and counting commenced the day following the placement of the individuals in the thermal gradient. The positions of the individuals were recorded hourly from 10:00 a.m. to 6:00 p.m. Prior to animal observation, the value displayed by the thermometers were recorded. Then, a snapshot of the animals inside the tank was taken using a Nikon d5200 digital camera. Snapshots were analyzed using ImageJ software to obtain the precise animal's location inside the tank (ver 1.8.0).

2.4. From animal locations to temperature values

Before converting the animals' locations into temperature values, we checked the linearity of the temperature gradient for each set of observations using the "lm" function in R 4.1.2 (R Development Core Team, 2022). Once the temperature linearity was confirmed, the individuals' location data were converted in a linear scale to temperature by interpolating temperature (namely T_1 and T_2) and location (namely x_1 and x_2) values between two adjacent thermometers. The linear equation was calculated according to Eq. (1):

$$\begin{cases} T(x) = \frac{B \cdot x}{A} - \frac{B \cdot x_1}{A} + T_1 \\ B = T_2 - T_1 \\ A = x_2 - x_1 \end{cases} \quad 1$$

where: x_1 and x_2 are the linear locations, expressed in cm, of the two adjacent thermometers; T_1 and T_2 are the temperature values, expressed in $^\circ\text{C}$, registered by the two adjacent thermometers; $T(x)$ is the interpolated temperature value at any location x between the two thermometers. To obtain the temperature corresponding to the animal location, the position of the animal in cm was assigned to the x parameter in Equation (1). Temperature data obtained by daily

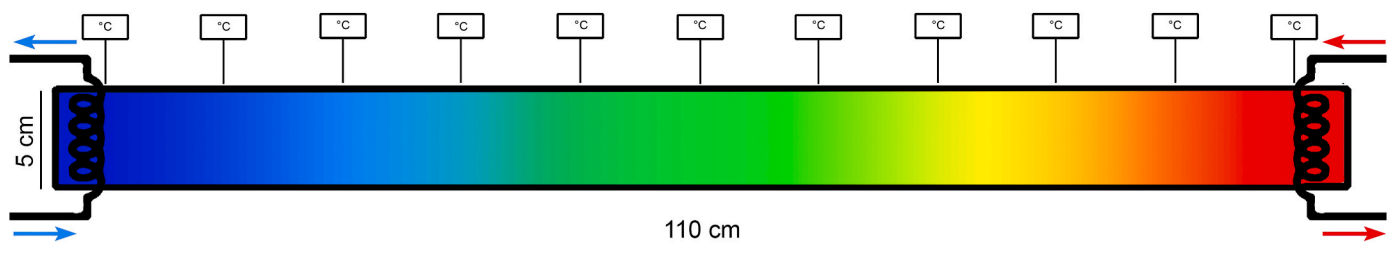


Fig. 2. Schematic diagram of the rectangular thermogradient apparatus. Blue – cold water at $7 \text{ }^\circ\text{C}$; Red – hot water at $23 \text{ }^\circ\text{C}$; Arrows – water flows from circulating water baths.

observations were clumped to fit a probability distribution plot of the temperatures, hereafter referred to as average percentage distribution (APD). APD kurtosis (i.e., the measure of the tailedness of the distribution, which is a proxy of how often outliers occur) was visually inspected daily.

2.5. Thermal ranges estimation

Within the daily APDs, we identified different thermal ranges according to Verbitsky et al. (2017, 2018).

- The Thermal Preferendum (TP) is defined as the interval of the APD at which 60% of the animals were located (temperature values between the 20th and the 80th quantiles).
- The Temperature of Normal Performance (TNP) is defined as APD interval where 90% of the individuals' observations were located (temperature values between the 5th and the 95th quantiles).
- The Avoided Temperature (AT) is the interval of the APD at which $\leq 10\%$ of the individuals' observations were located (temperature values below the 5th quantile and over the 95th quantile). AT represents the extreme temperature conditions at which the organisms can survive albeit with significantly reduced performance.

A schematic representation of the experiment, from snapshot acquisition to APD construction, is presented in Fig. 3.

2.6. Statistical analysis

The statistical differences between the six-day ADPs were initially assessed using an Anderson-Darling test for k-samples (Scholz and Stephens, 1987; H_0^k : the k-independent samples with sample sizes n_1 to n_k arose from a common unspecified distribution, $\alpha = 0.05$). Subsequently, pairwise comparisons between all possible combinations of daily ADPs were statistically tested using a two-sample permutational Kolmogorov-Smirnov test (Schröder and Trenkler, 1995; H_0^2 : the two samples come from the same distribution $\alpha = 0.05$). The choice of the permutation method for the p-values is guided by the fact that the p-value correction for multiple comparisons is unnecessary. Given the large number of comparisons needed, a classical p-value correction would have been an excessively conservative approach with a high likelihood of producing false negatives.

3. Results

At the end of the experiment, no dead individuals were recorded. The thermal gradient inside the tank maintained nearly constant linearity throughout the experiment (mean $R^2 = 0.75 \pm 0.03$; $p < 0.05$; Supplementary file_1: LM_summary). In total, 54 tank snapshots were acquired and 1620 location records of *N. longicaudatus* (Supplementary file_2) were converted in temperature values. Over the course of the six-day experiment, the kurtosis of the APD shifted from platykurtic to a more mesokurtic shape. Both the Anderson – Darling k-sample and the

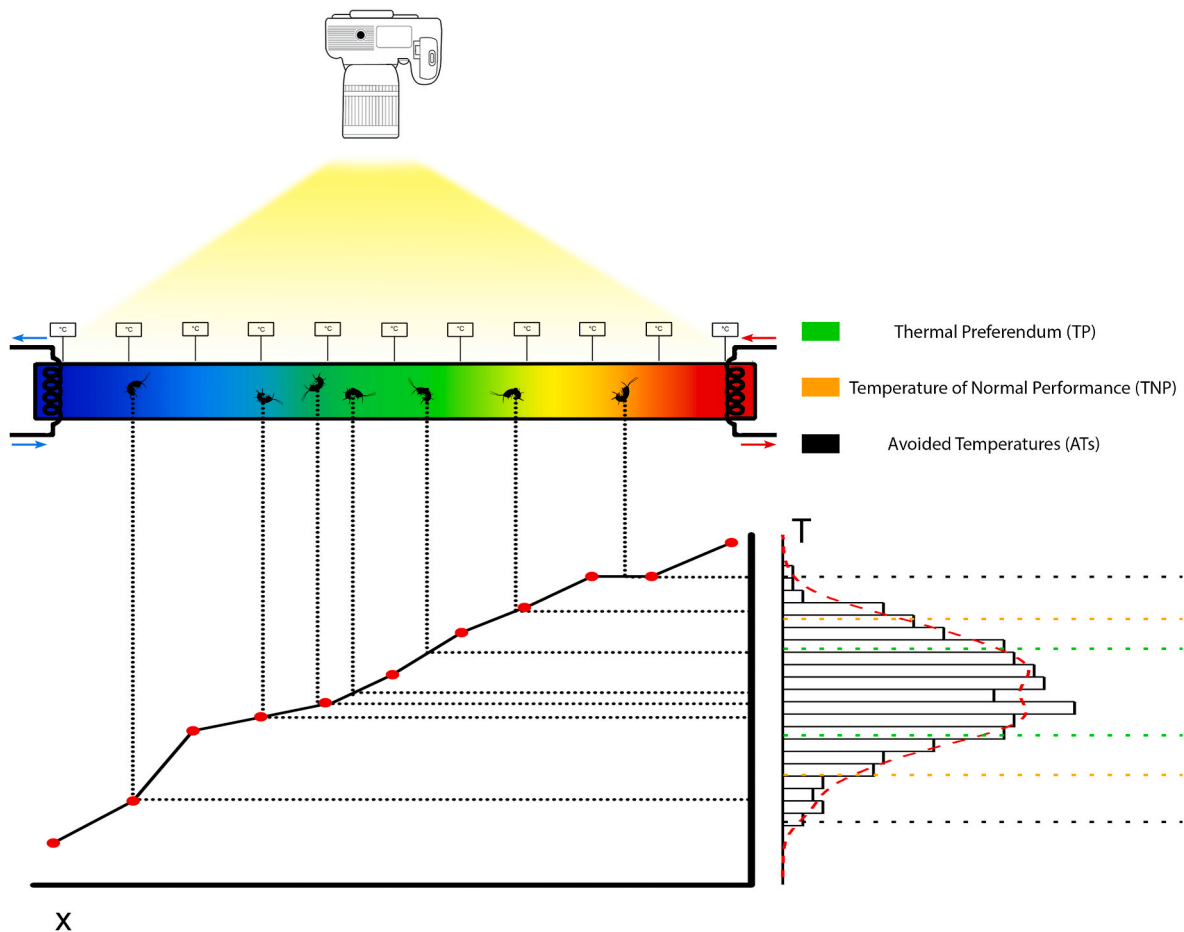


Fig. 3. Schematic representation of the experimental workflow implemented to determine the thermal range of the species *Niphargus longicaudatus*: snapshot acquisition of individuals' location along the horizontal temperature gradient (top left); conversion of individuals' location to temperature values (bottom left); estimation of the thermal average percentage of distribution (APD) (bottom right). Red dots – thermometers location and temperature; Red dotted curve – probability distribution curve; temperature interval between the green dotted lines on APD – Thermal Preferendum (TP); temperature interval between the orange dotted lines on APD – Temperature of Natural Performance (TNP); temperature interval between the orange and black dotted lines on APD - Avoided Temperatures (ATs).

pairwise permutational two-sample Kolmogorov-Smirnov tests indicated a clear statistical difference among the six days ADPs (Supplementary_file_3).

On day 1, the thermal range occupied by *N. longicaudatus* spanned from 8.66 ± 0.41 °C to 20.16 ± 0.89 °C, while, on day 6, the occupied thermal range was 10.86 ± 0.59 °C to 18.39 ± 0.92 °C (Fig. 4).

In detail, TP range shifted from 12.81 ± 2.19 °C - 18.59 ± 0.45 °C on day 1 - 12.64 ± 0.49 °C - 15.88 ± 0.55 °C on day 6. The TNP range also changed from 10.07 ± 1.14 °C - 19.39 ± 0.70 °C on day 1 - 11.58 ± 0.56 °C - 17.56 ± 0.55 °C on day 6. Regarding the AT range, the lower one went from 8.66 ± 0.41 - 10.07 ± 1.14 °C on day 1 - 10.86 ± 0.59 °C - 11.58 ± 0.56 °C on day 6, while the upper one dropped from 19.39 ± 0.70 °C - 20.16 ± 0.89 °C on day 1 - 17.56 ± 0.55 °C - 18.39 ± 0.92 °C on day 6 (Table 1).

4. Discussion

There are many ways to evaluate a species' thermal niche, including empirical studies conducted in the laboratory under controlled settings and field studies concerning the temperature regimes of the habitats where the species is found. Field studies can be biased by the sampling effort, which could not always be sufficient to identify the actual distribution of a species. This is a major impediment for groundwater obligate species because of the challenging access to subterranean habitats that prevents sampling in many parts of the subterranean environments (the so-called Racovitza impediment; Ficetola et al., 2019). Laboratory-controlled assays typically offer more trustworthy information. In this study, we examined the thermal niche of the widely-distributed groundwater amphipod species *N. longicaudatus* by monitoring, in a controlled thermal gradient, 30 adult individuals for six days. Our findings demonstrate that *N. longicaudatus* has a broad thermal niche with a natural performance range of 7–9 °C (depending on the day of observation), which is in line with the thermal regime of the distribution of this species complex (Ruffo and Stoch, 2005) in Italy. It is

Table 1

Thermal ranges of the APD (mean \pm SD; °C) calculated from field data and laboratory experiment. TP – Thermal Preferendum; TNP – Temperature of Natural Performance; AT – Avoided Temperature.

Day	TP	TNP	AT-left	AT-right
Day 1	12.81 \pm	10.07 \pm	8.66 \pm	19.39 \pm
	2.19–18.59 \pm	1.14–19.39 \pm	0.41–10.07 \pm	0.70–20.16 \pm
Day 2	0.45	0.70	1.14	0.89
	12.54 \pm	10.53 \pm	8.99 \pm	19.31 \pm
Day 3	0.43–17.93 \pm	1.34–19.31 \pm	1.01–10.53 \pm	0.44–20.69 \pm
	0.81	0.44	1.34	1.01
Day 4	11.94 \pm	10.77 \pm	9.35 \pm	19.15 \pm
	0.45–17.34 \pm	0.41–19.15 \pm	1.27–10.77 \pm	0.40–19.77 \pm
Day 5	0.69	0.40	0.41	0.44
	14.00 \pm	12.16 \pm	10.77 \pm	19.75 \pm
Day 6	1.26–18.72 \pm	1.70–19.75 \pm	2.04–12.16 \pm	0.56–20.08 \pm
	0.41	0.56	1.70	0.65
Day 6	15.88 \pm	13.54 \pm	10.71 \pm	18.38 \pm
	1.05–17.43 \pm	2.30–18.38 \pm	3.25–13.54 \pm	0.95–19.20 \pm
Day 6	0.57	0.95	2.3	1.21
	12.64 \pm	11.58 \pm	10.86 \pm	17.56 \pm
Day 6	0.49–15.88 \pm	0.56–17.56 \pm	0.59–11.58 \pm	0.55–18.39 \pm
	0.55	0.55	0.56	0.92

noteworthy that the temperature ranges occupied by the specimens during the test were markedly distinct from the mean annual temperature of the sampling site (9 ± 0.2 °C in the Stiffe Cave). Moreover, the final observed ranges of Thermal Preferendum (TP) and Temperature of Natural Performance (TNP) were notably wide, approximately spanning from 13.0 °C to 16.0 °C and from 12.0 °C to 18.0 °C, respectively. Our observations of substantial variations in daily ADPs and the absence of intra-specific predation are suggestive of a normal behavior, typical of healthy specimens. Given the limited trophic resources in cave habitats, obligate groundwater species are expected to engage in continuous exploration of the environment to seek sustenance. Aggressive behavior is replaced by a constant search for food, a phenomenon attributed to the

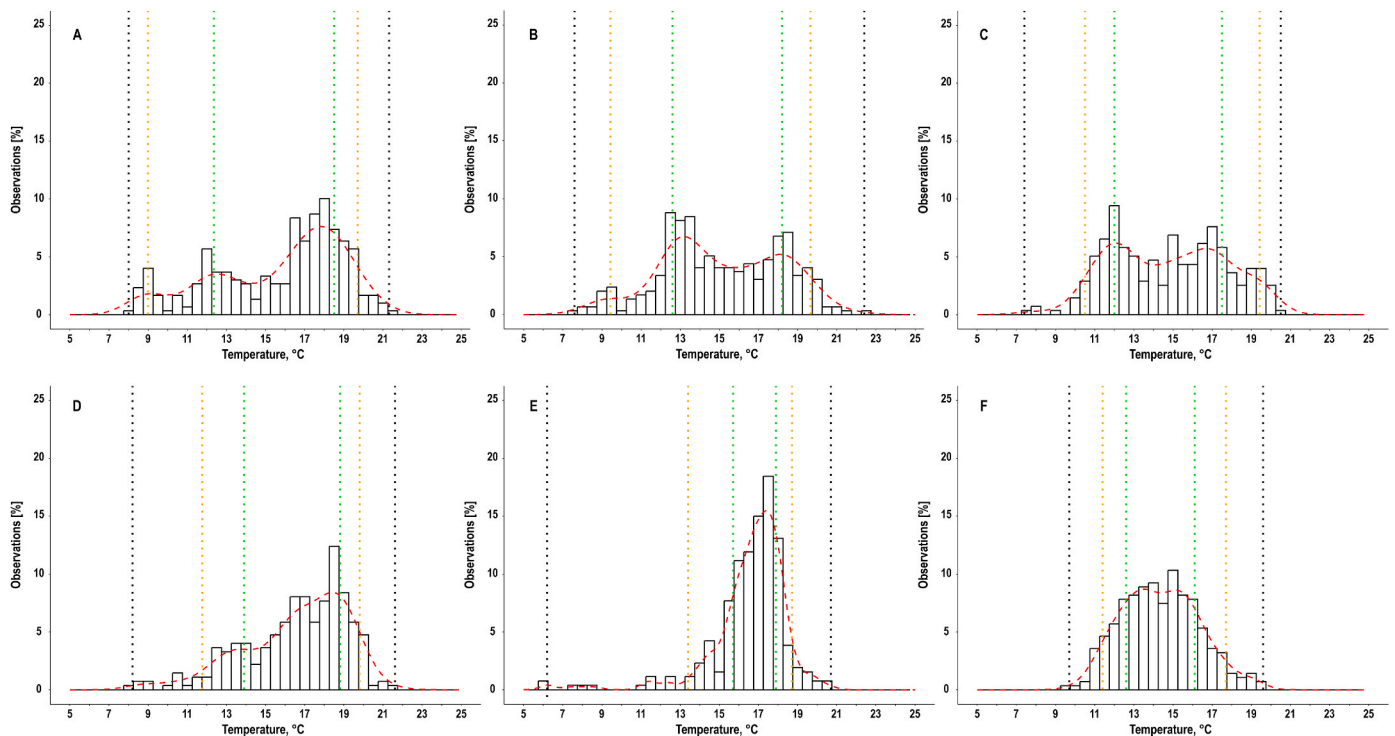


Fig. 4. Average Percentage Distributions (APD) of recorded *Niphargus longicaudatus* locations in the experimental thermal gradient. A - Day 1, B - Day 2, C - Day 3, D - Day 4, E - Day 5, F - Day 6 (final ADP). Y axis - percentage of records. X axis - temperature values in °C. Red dotted curve - probability distribution curve; temperature interval between the green dotted lines - Thermal Preferendum (TP); temperature interval between the orange dotted lines - Temperature of Natural Performance (TNP); temperature interval between the orange and black dotted lines - Avoided Temperatures (ATs).

activity modulation of serotonergic neurons in some cave species (Rétaux and Jeffery, 2023).

Arnscheidt et al. (2012) conducted a similar study using the chronic method experiment to investigate the thermal niche of the obligate groundwater species *Niphargus irlandicus* Schellenberg, 1932, and their findings were consistent with our results. Despite being sampled at temperatures ranging from 10 °C to 12 °C, the specimens displayed a thermal niche breadth of 0 °C–35 °C, with observed TP and TNP approximately ranging from 10.0 °C to 25.0 °C and from 5.0 °C to 30 °C, respectively. Similarly, Knight et al. (2015) reported comparable results regarding the thermal niche breadth of *Niphargus aquilex* Schiödte, 1855 and *Niphargus glenniei* (Spooner, 1952): the observed thermal ranges of the two species were markedly broader than the temperatures measured at the sampling sites (12 °C). Specifically, *Niphargus aquilex* showed a thermal niche width of 1.0 °C–26.0 °C, while *Niphargus glenniei* survived in a thermal range of 1.0 °C–24.0 °C. Issartel et al. (2005, 2007) conducted metabolic studies on *Niphargus rhenorhodanensis* Schellenberg, 1937 and found that this species also exhibited a broad thermal tolerance, from 3 °C to 17 °C. However, Colson-Proch et al. (2009) demonstrated that, despite its wide thermal niche, *N. rhenorhodanensis* has poor acclimation ability to fast thermal increase: a heat shock of +6 °C represents an important thermal stress for this species. These results suggest that the thermal tolerance of *Niphargus* species is likely influenced by the entity of thermal fluctuations in their habitat, aligning with the previously proposed hypothesis for subterranean beetles by Colado et al. (2022).

Since many groundwater habitats are characterized by thermal inertia (Marmonier et al., 2023), it is expectable that natural selection has favoured ectothermic species less able to cope with thermal values outside of their narrow thermal optimum (Issartel et al., 2007). Nevertheless, our findings, alongside the results obtained from the above-discussed studies, challenge the long-standing notion (Chappuis, 1927) that obligate groundwater species are all exclusively cold-stenotherms adapted to narrow temperature ranges.

Despite more than a century of scientific inquiry (started with Thienemann, 1908), the nature of the thermal adaptation of the *Niphargus* species remains a topic of ongoing debate and has yet to be fully clarified. To explain the potential eurythermic nature of some *Niphargus* species, some authors have put forth hypotheses that invoke paleogeographic, behavioral, and physiological processes. At the beginning of the 20th century, Thienemann (1908) classified *Niphargus* species as remnants of the last glacial maximum and, consequently, hypothesized that they would be stenothermic organisms with a preference for cold environments. However, about fifty years later, Ginet (1960) challenged this idea by observing that *Niphargus* species could survive at temperatures ranging from 0 °C to 24.5 °C, with a TP range of 8 °C–14 °C. Besides shielding these animals from climate change, the wide thermal tolerance found in several *Niphargus* species represents a beneficial trait for the recolonization of resource-rich but less thermally stable surface water habitats (Copilaş-Ciocianu et al., 2018). The findings of this study lend support to the idea that groundwater colonization is not an evolutionary dead-end, at least for some species (Stern et al., 2017). For the same reason, species with broad niche breadths are more capable to colonise other groundwater habitats, thus coping with thermal conditions that may be unsustainable by their populations in the long term.

Several researchers have suggested that the long-term changes in groundwater conditions that occurred at the geological time scale provided a range of climatic conditions to which the fauna adapted over time (Maurice and Bloomfield, 2012; Knight et al., 2015). Conversely, other authors have argued that the inhabitants of thermally stable environments, such as groundwaters, have not evolved highly temperature-sensitive organs to detect temperature changes because they have adapted to a thermally stable environment. Thus, they do not actively avoid potentially detrimental thermal conditions because they are not able to properly perceive them (Arnscheidt et al., 2012). On the

other hand, it is also possible that many *Niphargus* species are exceptional “conformers”, meaning that they have developed behavioral and metabolic adjustment mechanisms that allow them to maintain their fitness throughout a wide range of temperatures (Dillon et al., 2009). Accordingly, Arnscheidt et al. (2012) and Briellmann et al. (2011) observed a near-total reduction of the swimming activity for the species *N. inopinatus* Schellenberg, 1932 and *N. irlandicus*, respectively, when these animals ventured into the colder zones of the thermogradiant apparatus. Similarly, Issartel et al. (2005; 2007) reported a marked decrease in oxygen consumption rates at temperatures below 8 °C for *N. virei* Chern and Beutler, 1976 and *N. rhenorhodanensis*. Another key factor may be the expression of the so-called ‘metabolic key’ enzymes, whose expression in eurythermal ectotherms tends to increase when exposed to thermal conditions close to the outer limits of avoided temperatures (Issartel et al., 2005; Somero, 2004; Zakhartsev et al., 2004).

Despite the fact that our findings and those from earlier studies point to a wide temperature niche for some *Niphargus* species, we avoid drawing premature conclusions. First, not all the obligate groundwater *Niphargus* species have the same thermal tolerance, and broad thermal niche breadths. Second, several species for which experimental tests have been performed may live in different groundwater habitats, from the more variable shallow subterranean habitats where temperature may fluctuate significantly along the year to the deepest saturated karst where thermal stability is the rule; therefore, these species could have a higher thermal plasticity. Third, when it comes to groundwater obligate species, laboratory studies aimed at assessing their thermal niche encounter other impediments (Di Lorenzo et al., 2019). Groundwater species do not reproduce in the laboratory (Di Lorenzo et al., 2019). The population density of organisms that have adapted to living in groundwater is frequently low, so it is often challenging to recruit enough specimens for experiments (Mammola et al., 2019). In addition, laboratory conditions, though accurate, never are identical to the field condition where factors such as humidity, seasonality, interspecific interactions, ontogenetic stages and food availability might have a role in determining the thermal tolerance of a species (Copilaş-Ciocianu and Borş, 2016; Mammola et al., 2019b). For instance, in our experiment, the specimens of *N. longicaudatus* were deprived of food during the six days of observation. Food shortage is a potential environmental cue of dispersal for many groundwater organisms (Malard et al., 2023a,b). Wandering for food may explain why we assessed platykurtic APD in the early days of our study and a mesokurtic shape at the end of the trials, when the animals may have monopolized their reserves to resist food shortages on the place until conditions become favorable again, thereby avoiding any dispersal hazards (Malard et al., 2023a,b).

Thermal acclimation is fundamental for the obligate groundwater dweller amphipods, because they have poor dispersal capacity. The thermal plasticity of some species may allow habitat shifts in thermally variable habitats, giving potential for survival and reproduction, as shown in other obligate subterranean species (Pallarés et al., 2021).

5. Conclusion

Our study provides new insights into the thermal preferences of the groundwater amphipod *Niphargus longicaudatus*, highlighting its ability to survive in a wide range of temperatures, significantly deviating from those observed at the sampling site. Our findings support the idea that some obligate groundwater species could have evolved mechanisms to cope with temperature fluctuations, allowing them to maintain their fitness even in adverse thermal conditions. Furthermore, our results emphasize the importance of studying the thermal niche of species as a key factor in understanding how organisms respond to climate change. Future studies on the thermal ecology of groundwater species should consider paleogeographic events, behavioral and metabolic mechanisms that contribute to shaping the thermal preferences of these animals. Along with contributing to a better understanding of the thermal ecology of the obligate groundwater amphipod species *N. longicaudatus*, our

study provides information for the development of conservation strategies for these ecologically and biologically important organisms.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Other (please explain: e.g. ‘I have shared the link to my data as an attachment’).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2023.103654>.

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