Limited differentiation of fundamental thermal niches within the killifish assemblage from shallow temporary waters

Jakub Žák[a,b], Martin Reichard[a], Lumír Gvoždík[a,*]

[a] The Czech Academy of Sciences, Institute of Vertebrate Biology, Květná 8, 60365 Brno, Czech Republic
[b] Department of Zoology, Faculty of Science, Charles University, Viničná 7, 12800 Prague, Czech Republic

ABSTRACT

The coexistence of ectothermic species is enabled among other factors by the differentiation of their thermal niches. While this phenomenon is well described from deep temperate lakes, it is unclear whether the same pattern applies to temporary pools. In this study, we examined fundamental thermal niches in three coexisting annual killifish species *Nothobranchius furzeri*, *N. orthonotus* and *N. pienaari* from temporary pools in southern Mozambique. We hypothesized that the disparate thermal requirements of the three congeneric species are a candidate niche component that facilitates their local coexistence. We estimated species' thermal requirements as preferred body temperatures (T_{pref}) in a horizontal thermal gradient. Under thermal gradient conditions, sympatric killifish maintained their body temperatures within similar T_{pref} ranges despite some variation in mean T_{pref}. The daily variation in water temperature in their native habitats enables killifish to thermoregulate at least for part of the diurnal cycle. We conclude that the coexistence of African annual killifish species is possible without the differentiation of their fundamental thermal niches.

1. Introduction

Environmental temperature is one of major factors determining species occurrence in a given habitat. Although environmental temperature represents a stress factor rather than a resource (Chase and Liebold, 2003), thermal requirements constitute an important part of a species' ecological niche because of the pervasive effect of temperature on organismal functions and processes (Magnuson et al., 1979). Implicit in the recent definition of ecological niche (Chase, 2011), thermal niche is the range of body temperatures at which positive population growth occurs (Bennett and Lenski, 1993; Gvoždík, 2018). This pertains mainly to ectotherms, because their body temperatures are directly influenced by environmental (operative) temperatures. To maximize population growth, ectotherm target body temperatures should be readily attainable in a given habitat. However, species occupying the same area often vary in their thermal niches (Magnuson et al., 1979). Although knowledge of organisms' thermal niches provides invaluable information for fundamental ecological issues, such as the impact of climate change on ectotherm populations (Dulvy et al., 2008) and the potential coexistence of ecologically similar species (Ding et al., 2018; Ohlberger et al., 2008) thermal niche variation within ecological communities is not fully understood.

Several scenarios may explain thermal niche differentiation ranging from interspecific competition for shared limited resource(s), through past competitive exclusion of species with similar requirements and divergent selection to the recent occupation of the same area by species with disparate thermal requirements (Paterson and Blouin-Demers, 2017). Thermal niche differentiation is well known from various terrestrial and aquatic communities (Ding et al., 2018; Magnuson, 2010; Mushinsky et al., 1980). Deep temperate lakes are particularly illustrative of this phenomenon, because fish species are separated across the water column according to their thermal requirements (Larsson, 2005; Magnuson, 2010; Ohlberger et al., 2008). In contrast to lakes, information about thermal niche differentiation in shallow water bodies is scarce.

Shallow water bodies provide a thermally challenging environment to ectotherms due to both high daily temperature fluctuations (Heath et al., 1993; Reichard et al., 2009) and the occurrence of extreme temperatures, i.e. above 40 °C (Heath et al., 1993; Podrabsky et al., 1998; Reichard, 2016). The temperature range may reach 15–20 °C between midday and early morning (Podrabsky et al., 2008; Reichard et al., 2009). A depth of as little as 15 cm contains sufficient variation for daytime thermal stratification (Hadamová and Gvoždík, 2011). The difference between bottom and surface temperature may be as much as 15 °C, providing the opportunity for behavioral thermoregulation in aquatic organisms (Hadamová and Gvoždík, 2011). In comparison to

* Corresponding author.
E-mail address: gvozdik@brno.cas.cz (L. Gvoždík).

large water bodies, the opportunity for behavioral thermoregulation in temporary pools is more time-limited, potentially increasing inter-specific competition for space at suitable water temperatures. Accordingly, this might lead to thermal niche differentiation as commonly observed in lakes (see references above).

In this study, we examined the fundamental thermal niches of three coexisting species of *Nothobranchius* spp. from southern Mozambique. Fundamental thermal niche was estimated using the optimality paradigm (MacArthur and Pianka, 1966; Schoener, 1971; Stephens and Krebs, 1971). This assumes that under the absence of any biotic and abiotic limitations, ectotherms are able to maintain body temperatures that maximize population growth, i.e. preferred body temperatures. The three species, *N. furzeri, N. orthonotus* and *N. pienaari* are closely related (Dorn et al., 2014) and their distributions strongly overlap (Reichard et al., 2017b). They commonly occur in a single pool with subtle microhabitat preferences and high trophic niche overlap (Polačik et al., 2014; Polačik and Reichard, 2010; Reichard et al., 2017b, 2009). Accordingly, we hypothesized that disparate thermal requirements among the three congeneric species are a candidate niche component that facilitates their local coexistence. To test this hypothesis, we compared T_{pref} ranges not only among species, but also with temperatures that fish potentially experience in their native habitats, i.e. the opportunity for behavioral thermoregulation (Hadamová and Gvoždík, 2011).

2. Materials and methods

2.1. Study species and maintenance

Nothobranchius furzeri, N. orthonotus and *N. pienaari* are small (4–8 cm) cyprinodontiform fishes inhabiting ephemeral savanna pools in southern Mozambique. They are primarily differentiated by male nuptial coloration (Wildekamp, 2004), with ecological differentiation from a generalized form restricted to a limited number of species (Costa, 2018). The separation of the three species was estimated to be 3–4 mya (Dorn et al., 2014), which is probably sufficient time for any thermal niche divergence to occur.

We used populations of *N. furzeri* (population MZCS 002 after Bartáková et al., 2015), *N. orthonotus* (MZCS 002) and *N. pienaari* (MZCS 505) from the center of the area where all three species are sympatric. All study populations originated from the same region (the northern bank of the River Limpopo, Gaza province, Mozambique). Experimental individuals were 3rd to 4th generation descendants of wild-caught fish that were bred in large captive populations to minimize inbreeding (Blažek et al., 2016). All generations were kept at the breeding facility of the Institute of Vertebrate Biology in Brno, Czech Republic.

Experimental fish were hatched on 1 August 2016 according to the standard protocol for *Nothobranchius* species (Polačik et al., 2016). Fish were housed in 60 L aquaria with approximately 30 individuals of both sexes. Some males of the most aggressive species, *N. orthonotus,* were kept separately from females to prevent male-male aggression, female harassment, and potential injuries. Fish were fed once per day ad libitum with frozen bloodworms (chironomid larvae). The light regime was set to 14 L:10D. Aquarium water temperature was maintained at 26 ± 1 °C. This water temperature was chosen because it represents the typical mean ambient temperature in natural pools. Water (one third of total volume) was changed twice per week.

For the purpose of this study, we used a total of 30 adult individuals per species (Table 1). Once they reached the age of 11 weeks, fish were transported in groups of 12–15 (a combination of all species and sexes) to the experimental facility of the Institute of Vertebrate Biology in Studenec, Czech Republic. In the experimental facility, three to five individuals of the same species were kept in 20 L tanks. The thermal, light, and feeding regimes were identical to those in the breeding facility. Fish were kept under these conditions for at least two days before

Table 1

Sample size and body mass (mean (SD)) of three *Notobranchius* species used in this study.

| Species	Sample size		Body mass (g)	
	Females	Males	Females	Males
N. furzeri	15	15	0.83 (0.13)	1.74 (0.85)
N. orthonotus	19	11	0.94 (0.30)	1.15 (0.45)
N. pienaari	15	15	0.31 (0.05)	0.66 (0.11)

measurements. All experimental data were collected between 20 October and 19 November 2016.

2.2. Preferred body temperatures

We measured preferred body temperature (T_{pref}) in a horizontal thermal gradient (15.5–38.5 °C) maintained in a stainless steel tank (240 × 60 × 60 cm). The tank was divided into three lanes. Each lane had 12 partially separated compartments (20 × 20 cm) that varied in water temperature. Partitions were made of 0.5 cm styrofoam with a 3 × 3 cm opening to enable fish movement between compartments. The water temperature increased by 2.0 ± 0.5 °C with each compartment along the horizontal gradient. Water temperature was maintained by the combination of Peltier modules mounted under the bottom of each compartment together with an aquatic heat recuperation system connected to a programmable control unit (see Gvoždík, 2003 for further details). Each compartment was gently aerated to prevent water temperature stratification. Temperatures were measured using calibrated thermistor probes connected to dataloggers (resolution 0.01 °C; UX120-006M, HOBO, Onset Computer, Bourne, MA, USA) three hours before the start of recording and again no longer than one hour after terminating a trial. The mean temperature calculated from both values was used as the representative water temperature for each compartment. After a trial, the tank was re-filled with non-chlorinated well water to the depth of 3 cm. The room temperature was kept at 26 ± 1 °C to maintain the stability of the thermal gradient during trials.

We placed three randomly chosen fishes separately into one lane of the thermal gradient for 15 h before the start of trial. Each fish was placed into the compartment with the same water temperature as in the stock aquarium. Fish motor activity was recorded during the following day, between 10:00 and 17:00, using a digital camera surveillance system (12 fps; MTV-63S80H-A-ICR-R, Mintron, Taipei, Taiwan). This time window matches the period when natural pools become thermally stratified and fish have an opportunity to thermoregulate (Fig. 1). Fishes were not fed during the experimental trial, because a pilot study confirmed that feeding had a slight influence on T_{pref} in each species. After the trial, each fish was weighed to the nearest 0.01 g (Kern EG, Kern, Balingen, Germany). All fishes were used only once in the experiment and returned back to the breeding facility for stock maintenance after measurement.

From the videos, we recorded the horizontal positions of fishes in the gradient at 10 min intervals. Average T_{pref} over six intervals each hour was used for statistical analysis. The body temperature of small fish rapidly converges with that of the surrounding water (Lutterschmidt and Hutchison, 1997), and thus we estimated fish body temperatures by extrapolating from water temperature at given horizontal positions. The lower (LBT_{pref}) and upper (UBT_{pref}) boundaries of preferred body temperature were calculated from individual T_{pref} distribution as the 10th and 90th percentile, respectively. We assume that these values delimit the target range of T_{pref} more accurately than the narrower or parametric estimates (Hertz et al., 1993; Magnuson et al., 1979). In addition, thermal dependence of the key physiological process, aerobic scope, supports a wider rather than narrower estimate of T_{pref} range (Gvoždík and Kristín, 2017). The range of preferred body temperatures was calculated as the difference between UBT_{pref} and
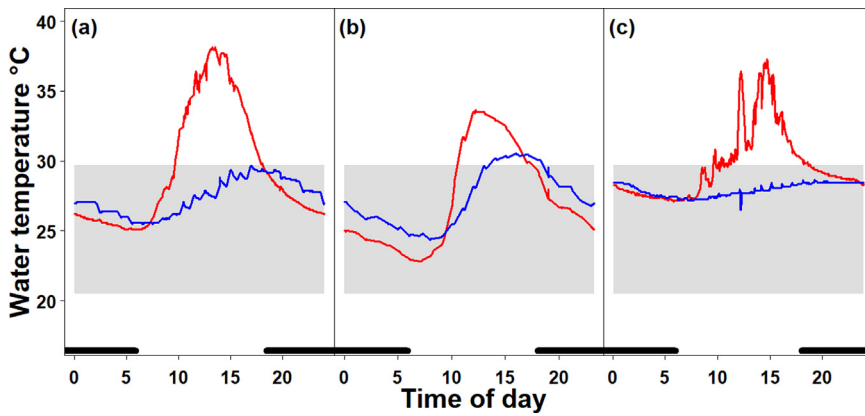
Fig. 1. Diel variation in surface and bottom water temperatures in three pools with *Nothobranchius* spp. in Mozambique. The grey area represents the range of preferred body temperatures averaged across three species, *N. furzeri, N. orthonotus,* and *N. pienaari,* given only minor interspecific variation in this trait (see Fig. 2). Horizontal black bars denote the night period. (a) Maximum depth 35 cm, (b) maximum depth 20 cm with canopy cover, (c) maximum depth 95 cm.

LBT_{pref} (i.e. central 80% of all measurements). For comparative purposes, we repeated our analyses with T_{pref} boundaries calculated as 25th and 75th percentiles.

The motor activity of each fish was recorded over seven 10 min intervals during trial and the number of times a fish crossed between compartments was summed for activity analysis. Four individuals (*N. furzeri* two females and one male; *N. pienaari* one male), which swam continuously across thermal gradients during the whole trial, and one inactive individual (*N. pienaari* male) were discarded from further analyses.

2.3. Field water temperatures

To obtain information about the daily temperature range that fish experience in the wild, we recorded water temperatures at three water bodies (Fig. 1) with the co-occurrence of at least two killifish species using temperature data loggers (two per site; HOBO, UA-002–64, Onset Computer, Bourne, MA, USA). The first datalogger was placed at the maximum depth on the bottom. The second datalogger was anchored 5 cm under water surface and shaded from direct sunlight. Temperatures were recorded at 1 min intervals over a 24 h period. Using these values and fish T_{pref} ranges, we calculated the opportunity for thermoregulation, which is the proportion of daytime that allows thermoregulation, i.e. maintaining body temperatures within the T_{pref} range, if the range of vertical thermal gradient > T_{pref} range (Hadamová and Gvoždík, 2011).

2.4. Statistical analyses

We applied a linear mixed model to analyze the effect of species identity on T_{pref}. The full model contained three fixed effects: species, sex, and experimental lane. Body mass, activity and time of day were added as covariates. The interactions between factors were also included. Random factors included both "individual identity" (intercept) and "time" (slope). We used a model simplification approach; the minimal adequate model was selected according to the lowest Akaike Information Criterion corrected for small sample size (AICc).

The ranges of T_{pref}, UBT_{pref} and LBT_{pref} were analyzed using a general linear model. The full model contained five factors: species, sex, body mass, gradient lane, mean T_{pref}, and species × sex interaction. The most parsimonious model with the lowest AICc was chosen by backward selection. In all models, the distribution of residuals was checked via diagnostic plots. Tukey *post hoc* tests were used for all interspecific comparisons. Fish motor activity was analyzed with a generalized linear model with a quasi-Poisson distribution because of high overdispersion. The full model contained three factors: species, sex, and gradient lane, and their interactions. Body mass was added as a covariate. All statistical analyses were performed in R using the "nlme" (Pinheiro et al., 2016), "MuMIn" (Bartoń, 2016) and "lsmeans" (Lenth,

2016) packages.

3. Results

In total, we obtained 591 T_{pref} measurements from 85 individuals of three species (five individuals were excluded, see above). The species varied in their mean T_{pref} ($F_{2,82} = 10.52$, $P < 0.001$; Fig. 2). Specifically, *N. orthonotus* maintained higher mean T_{pref} than both *N. furzeri* (Tukey HSD test, $P = 0.03$) and *N. pienaari* ($P < 0.001$), while the latter two were similar ($P = 0.08$). Generally, individual mean T_{pref} decreased with locomotor activity level ($F_{1,498} = 45.74$, $P < 0.001$). Individual identity (random intercept) and individual variation in the time course of body temperatures (random slope) explained 88% of total variation in T_{pref}.

The T_{pref} range was similar across the species (Fig. 2). Active individuals had a broader T_{pref} range than less active individuals ($F_{1,79} = 8.33$, $P = 0.005$). At the individual level, LBT_{pref} and UBT_{pref} were positively related to mean T_{pref} (LBT_{pref}: $F_{2,81} = 137.55$, $P < 0.001$; UBT_{pref}: $F_{2,81} = 132.07$, $P < 0.001$). Therefore, we used mean T_{pref} as a covariate to analyze the influence of species identity on these traits. The T_{pref} range boundaries were consistent among species (LBT_{pref}: $F_{2,81} = 1.78$, $P = 0.17$; UBT_{pref}: $F_{2,81} = 1.88$, $P = 0.16$; Fig. 2). Qualitatively identical results were obtained for T_{pref} boundaries calculated as 25% and 75% percentiles (Table S1, Fig. S1).
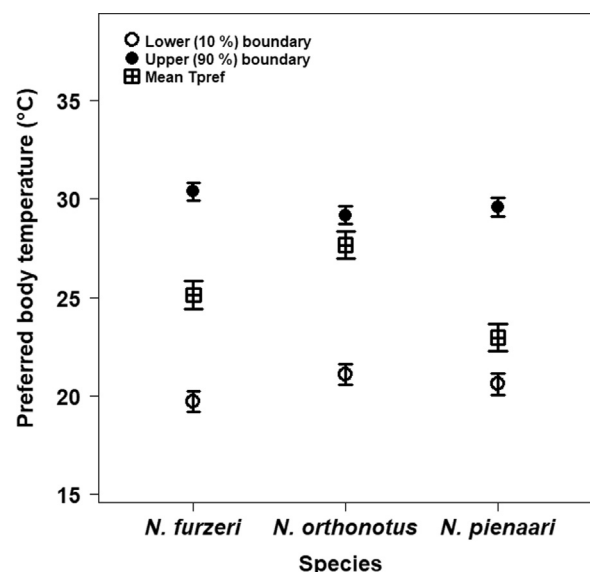
Fig. 2. Mean preferred body temperature, lower, and upper boundaries of preferred body temperature range in three *Nothobranchius* species. Boundaries were calculated as 10th and 90th percentile of individual body temperature distribution. Means (± SE) are model-estimated parameters.
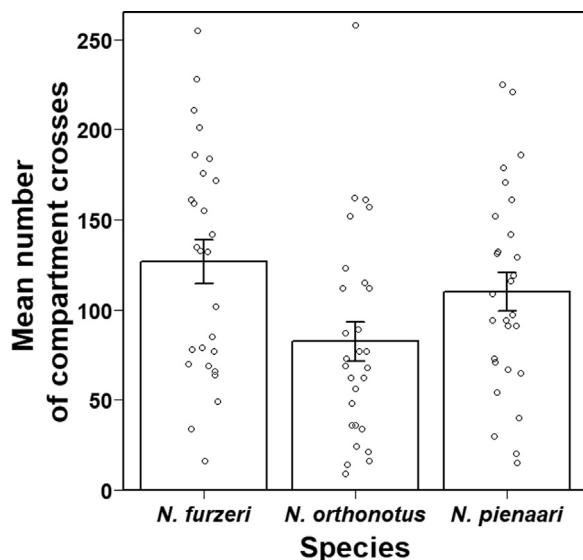
Fig. 3. The motor activity of three *Nothobranchius* species in a thermal gradient. Activity was estimated as the sum of compartment crosses over seven 10 min intervals. Data points were jittered horizontally to improve their visibility. Error bars represent 1 SE.

The index of locomotor activity varied among species ($\chi^2 = 7.82$, $P = 0.02$; Fig. 3). Specifically, *N. furzeri* was more active than *N. orthonotus* ($z = 2.74$, $P = 0.02$). The locomotor activity of *N. pienaari* did not differ from the other two species (NP vs. NF: $z = 0.99$, $P = 0.59$; NP vs. NO: $z = 1.79$, $P = 0.17$).

The daily temperature range in all temporary pools enabled killifish to thermoregulate for at least part of the day. In two deeper pools, killifish could behaviourally thermoregulate for 7–8 h per day (31–32% of the diurnal cycle; Fig. 1a, c). In the shallowest pool, thermoregulation was possible for 2.5 h only (10% of the diurnal cycle; Fig. 1b).

4. Discussion

Thermal niche often varies among species occupying the same area (Magnuson et al., 1979; Ohlberger et al., 2008). In contrast to these findings, our results show that the coexistence of African annual killifish species is possible without the differentiation of their fundamental thermal niches. In the laboratory thermal gradient, the killifish species maintained their body temperatures within the same ranges, though their mean values varied. We found a high level of variation in T_{pref} among individuals, which was related to their locomotor activity within the gradient. Below, we discuss these findings within the context of annual killifish ecology and the methodology of T_{pref} measurements.

Similarities in fundamental thermal niches suggest that (i) the killifish species were equally adapted to thermal conditions in temporary pools or (ii) their thermal niches were inherited from a common ancestor 3–4 mya (Dorn et al., 2014). On the one hand, the latter possibility seems surprising, because annual killifishes are a fast evolving species (Reichwald et al., 2015; Valenzano et al., 2015), and this time period should be sufficient for thermal niche differentiation. On the other hand, T_{pref} is often conserved within genera (Angilletta and Werner, 1998; Youssef et al., 2008; but see Labra et al., 2009). Specifically, the evolutionary rate of the upper T_{pref} boundary is relatively slow (Gvoždík, 2015), which may prevent thermal niche differentiation by increasing UBT_{pref}, even when diel thermal profiles in their native habitat provide the potential for it (Fig. 1). The limited thermal niche differentiation among annual killifish seems even weaker than trophic niche partition (Polačik et al., 2014; Polačik and Reichard, 2010), which concurs with the general ecological similarity of these species (Wildekamp, 2004). Hence, further studies will investigate whether the

co-occurrence of these taxa is determined by their differentiation in other niche dimensions, ecological trade-offs (Kneitel and Chase, 2004), or negative frequency dependence (Svensson et al., 2018).

Alternatively, because our fish were kept in stable temperature for 3–4 generations after import from the wild, we cannot rule out that this approach reduced intra- and interspecific variation in T_{pref} due to a rapid evolutionary response to the captive environment (Christie et al., 2012; Araki et al., 2007). However, as we mentioned above, the T_{pref} range seems a phylogenetically conservative trait (Gvoždík, 2015). Hence, we assume that our results were unbiased by captive rearing. Untangling the interactive effect of genetic, developmental, and environmental factors on T_{pref} variation in this system provides an interesting research agenda for further studies.

The T_{pref} range of the species we examined was almost identical to that previously observed in a South American annual killifish, *Austrofundulus limnaeus*, inhabiting thermally similar habitats (Podrabsky et al., 2008). A likely factor contributing to the wide T_{pref} ranges in these taxa is unpredictable thermal variation, and accordingly the opportunity for thermoregulation (Hadamová and Gvoždík, 2011), in their native habitat both within and among generations (Gilchrist, 1995; Reichard et al., 2017a). Under these conditions, the wide T_{pref} range may reduce the time and energy spent in behavioral thermoregulation (costs of thermoregulation), which can be invested in other activities such as feeding or reproduction (Angilletta, 2009; Huey and Slatkin, 1976).

Despite negligible variation in T_{pref} range, mean T_{pref} varied among species. Although all three species moved across the same temperatures in the thermal gradient, *N. orthonotus* spent more time at warmer temperatures than *N. furzeri* and *N. pienaari*. The cause of this variation is unknown. Perhaps because the T_{pref} range provides the best thermal compromise for various biochemical processes and physiological functions within an organism (Cossins and Bowler, 1987; Gvoždík and Kristín, 2017; Hochachka and Somero, 2002), variation in mean T_{pref} may reflect interspecific variation in acute demands to maximize performance of some physiological traits over others. In addition, the high individual variation in T_{pref} and the non-random distribution of "low-"and "high-T_{pref}" individuals among species may also contribute to this result. Anyway, this finding clearly demonstrates that characterizing T_{pref} using only its mean value may produce misleading information about the magnitude of intra- and interspecific variation in this trait.

During T_{pref} trials, locomotor activity level varied among killifish species. Individuals that maintained higher body temperatures increased their activity relative to individuals with lower T_{pref}. A similar relationship between activity and T_{pref} has been reported in other fish species, *Danio rerio* and *Oreochromis niloticus* (Cerqueira et al., 2016; Rey et al., 2015). This pattern may be related to "thermal-behavioral syndrome" (Michelangeli et al., 2017), which predicts that the T_{pref} of each individual fits somewhere within the cold–hot continuum and the position on this continuum directly influences individual personality, e.g. activity level, boldness, and accordingly habitat utilization. High (88%) individual variation in T_{pref} also concurs with this prediction. This individual variation in T_{pref} may be beneficial for reducing intraspecific competition in the wild (Beitinger and Magnuson, 1975; Medvick et al., 1981). Given the similar thermal requirements among taxa, individual T_{pref} variation may reduce not only intra- but also interspecific competition within the African annual killifish assemblage.

In conclusion, we showed that syntopic coexistence of three annual killifish is possible without differentiation in their fundamental thermal niche. Their wide T_{pref} ranges seem well suited to life in thermally heterogeneous temporary pools in the African savanna. However, a wider comparative study including more taxa distributed across a latitudinal gradient will help to resolve whether similar thermal requirements result from evolutionary conservation of this trait (Gvoždík, 2015; Muñoz et al., 2014) or adaptation to thermal conditions in their common habitat (Labra et al., 2009). Next, we demonstrated that focusing solely on mean T_{pref} may produce misleading results about the

trait variation. Preferred body temperature has remained the most meaningful measure in thermal ecology for decades (Huey, 1982). However, their accurate estimates require not only controlling for many confounding factors (Hutchison and Dupré, 1992), but also choosing suitable parameters characterizing this trait. As has been shown before (Gvoždík, 2015), the T_{pref} range is much more informative than mean T_{pref}, especially from the perspective of thermal niches (Gvoždík, 2018). The most comprehensive approach to estimating T_{pref} should also include information about ectotherm locomotor activity in the thermal gradient, because it may be associated with T_{pref} parameters. Finally, though mean T_{pref}s concur with previously published general recommendations on *Nothobranchius* spp. husbandry (Dodzian et al., 2018; Genade, 2005; Polačik et al., 2016), our study provides the first information about the thermal biology of these species, which are currently used as models in aging research (Cellerino et al., 2016; Hu and Brunet, 2018).

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2018.10.015.

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