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Thermal relationships in the habitat use of *Bombina variegata* tadpoles

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Abstract. If ectothermic animals live in habitats where temperatures reach their upper thermal limit, global warming may be problematic, particularly if the animals cannot escape from their respective habitats. Tadpoles of the Yellow-bellied Toad, *Bombina variegata,* develop in small, temporary water bodies that are exposed to the sun and prone to short-term temperature fluctuation, extreme heat events, and high risk of desiccation. Thermoregulation should be essential for the successful development of the tadpoles because metabolism, developmental time, heat stress and death, and desiccation are temperature dependent. Thus, already small temperature differences within ponds may have important physiological and developmental consequences for tadpoles and should therefore influence their habitat use. We investigated the thermal relationships in the habitat use of *B. variegata* tadpoles in semi-natural outdoor experiments, providing a natural day/night cycle of temperature and light and natural temperature gradients and ranges. In experimental puddles, equipped with temperature loggers, we recorded the distribution of the tadpoles with time-lapse videos. We then compared their positions to the respective temperature data. The thermal relationships detected in our field experiment were similar to thermal preferences observed in previous laboratory experiments with artificial temperature gradients. We found the tadpoles more frequently in cooler zones when the water temperature was above their thermal preference range, and in warmer zones when water temperature was below. The number of tadpoles increased in the shallow and warmer water in the cool forenoon, and the deep and cooler water in the warm afternoon. At higher temperatures, tadpoles accumulated in small, shaded areas. Apparently, *B. variegata* tadpoles use even limited opportunities to compensate for temperature fluctuations to avoid heat stress.

Key words. Amphibia, Anura, Bombinatoridae, daily movement, ectotherms, ephemeral water, thermoregulation, thermal limits, thermotaxis, temperature stress, thermal preference, shade preference.

Introduction

Due to higher temperatures and the increased frequency and intensity of heat waves (IPCC 2023, WMO 2023), climate change will cause thermal stress to many species (Gunderson & Stillmann 2015, Murali et al. 2023). This will be especially problematic for ectothermic animals that live in habitats where temperatures are close to their upper thermal limit and from which they cannot escape i.e., aquatic species or life stages.

Like many other anuran species (Skelly 1997), Yellow-bellied Toads, *Bombina variegata* (Linnaeus, 1758) spawn in small, temporary, and sun-exposed water bodies (Niekisch 1995, Schlüppmann 1996, Barandun & Reyer 1998, Joly et al. 2011, Gollmann & Gollmann 2012, Cayuela et al. 2022). Temperature is critical in these environments, as small volume of water and high exposure to the sun increase the risk of desiccation. If metamorphosis is not completed in time, an entire cohort of tadpoles may face death (Barandun & Reyer 1997). Moreover, such temporary puddles are prone to high temperature fluctuations (Nadeau et al. 2022) and can quickly reach extreme temperatures which cause heat stress or death.

The survival and development of amphibians is temperature dependent (Barandun & Reyer 1997, Govindarajulu & Anholt 2006, Blaustein et al. 2010, Turriago et al. 2015). Ectotherms have species-specific thermal limits in which they can develop normally and for optimal development thermal preferences, which may vary by developmental stage (Van der Have 2002, Drakulić et al. 2020). Even slight temperature differences can influence physiology and development. Amphibian larvae developing outside an optimal thermal range may show reduced growth rates (Rühmekorf 1958a, Smith-Gill & Berven 1979), and higher temperatures reduce developmental time (Morand et al. 1997). For example, an increase in water temperature by 1 °C can reduce the time to complete metamorphosis by 4–5 days in *Bombina variegata* (Dittrich et al. 2016). Ectothermic animals mainly alter their body temperature through behavioural thermoregulation, enabling them to maintain temperature within critical limits for development, particularly under extreme thermal conditions (Leger & Mathieson 1997, Amat-Trigo et al. 2023).

However, small aquatic habitats tend to be thermally almost homogenous, providing only limited opportunities for thermoregulation (Seebacher & Franklin 2005). Nevertheless, even small differences in temperature should be used by tadpoles to compensate for temperature fluctuations and to reduce heat stress. Several experimental studies have described thermoregulatory behaviours of tadpoles (Lucas & Reynolds 1967, Casterlin & Reynolds 1978, Hutchison & Hill 1978, Dupré & Petranka 1985, Drakulić et al. 2017), including two studies on *B. variegata* tadpoles (Ruhmekorf 1958b, Drakulić et al. 2020).

Developmental plasticity under variable thermal conditions and preferences of *Bombina variegata* tadpoles of the population studied herein were investigated previously (Dittrich et al. 2016, Drakulić et al. 2020). Thermal preferences were experimentally determined using a temperature gradient ranging from ~ 14 to 31 °C. The median thermal preference was plastic and varied among tadpoles of different developmental stages, origins, or previous temperature experiences, ranging from 22.6 to 27.0 °C (see Drakulić et al. 2020). Our study aimed to determine whether these experimental results can be confirmed under a natural day-night cycle with respective temperature gradients and ranges. Consequently, we conducted field experiments in experimental puddles. We expected that tadpoles would use warmer zones when the water temperature dropped below their thermal preference range and cooler zones when it was above. We (1) recorded temperature fluctuation and ranges in our experimental puddles during day and night to determine whether tadpoles had a choice between different temperatures; (2) analysed the temperatures at the tadpoles' positions to reveal at which times and ambient temperatures, tadpoles use warmer or cooler zones, and how it relates to their thermal preference, and (3) determined the spatial tadpole distribution and compared it with the available spatial temperature distribution to investigate if tadpoles move to zones with preferable temperatures, which indicates active thermoregulation.

Materials and methods

We investigated thermal relationships in the habitat use of *B. variegata* tadpoles under semi-natural conditions. We tested 18 groups of six tadpoles each, for 24 consecutive hours in experimental puddles. The experiments were conducted between July 22 and August 15, 2022, at a sandstone quarry near Ebelsbach, Franconia, Bavaria, Germany. Tadpoles were collected from two puddles in the quarry that were about to desiccate and kept in a container replicating their natural habitat. The larvae were in Gosner stage 30–37 (Gosner 1960). The experiments were performed directly on-site and thus comprised a natural day/night cycle of temperature and light.

Two experimental puddles (plastic containers) were embedded in the ground directly adjacent to each other. The rectangular puddles (Fig. 1A) measured 112 × 26 × 17 cm (length × width × depth). Similar to the original puddles, they were positioned in a place exposed to the sun (sun from ~ 10 am to 6 pm). Both puddles comprised a water depth gradient ranging from 2 to 14 cm along their long side. Each container was filled with 28 l of water and 3 l of sand that covered the ground. Both containers were equipped with 14 temperature loggers each (Thermochron® iButtons© DS1922l, accuracy ± 0.5 °C; Embedded Data Systems, USA), distributed equally and recording temperatures in 5-minute intervals. Eight iButtons were placed next to the container walls on the ground at depths of 2, 6, 10 and 14 cm, respectively. The remaining six iButtons were fixed to swimming sticks, 5 mm below the water surface and above the iButtons in 6, 10 and 14 cm depth (Fig. 1A). Per experimental trial six tadpoles were put in each container and left alone for one night before starting an experiment. Then the tadpoles' positions were recorded using a camera in timelapse mode (action camera, version and stand: 20180323V3.3, China; 3840 × 2160 px, 2 frames s^{-1}). The camera was placed in a central position between and 65 cm above both containers. To allow observations at night (9/10 pm to 5 am), the containers were briefly (60 sec) illuminated every full hour with two lamps (LED, cold white, 50 W), controlled by a timer (Camway HM231UK, Hongkong). Electronic devices were powered using a car battery. The camera recorded permanently throughout an experimental trial. After each experimental trial, all tadpoles were put in a container for "tested" tadpoles, thus avoiding double testing

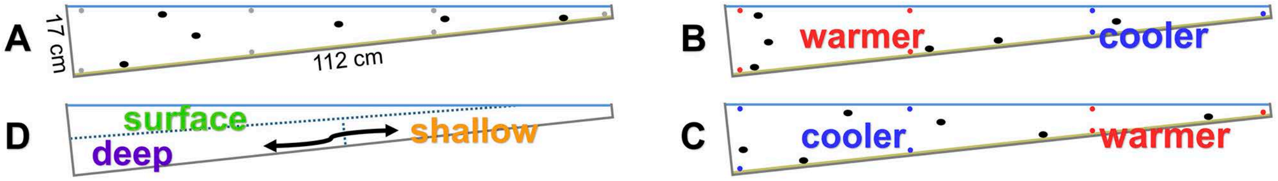

Figure 1. Schematic cross-section (lateral view) of an experimental puddle; (A) indicating the position of seven of the 14 temperature loggers (iButtons, grey dots), and indicated position of six tadpoles (black dots); (B, C) Examples illustrating the variable temperature distribution between warmer (red iButtons) and cooler (blue iButtons) zones; (D) the experimental puddle was imaginarily divided into three spatially distinct zones: shallow (orange), deep (purple), and a surface (green) water zone. The arrow indicates a movement example of tadpoles between these zones. Figures are not to scale.

of individuals, and later released in nearby puddles in the quarry. This procedure was repeated nine times, making a total of 18 experimental trials. For each experimental trial, the container was cleaned, and the sand and water were replaced. The videos were analysed hourly from 6 am (starting the day after an experiment was prepared) until 5 am the following day. At each hour the video was stopped (for night hours directly after the lights turned on), the location of each tadpole assessed and the nearest iButton identified and noted. When the sun was shining, the percentage of the shaded area was estimated (in 3% steps for shade not covering more than 9% of the container surface and in 10% steps for larger areas of shade), and it was noted how many tadpoles were in the sun or shade, respectively.

To identify the temperatures the tadpoles were exposed to, we measured the water temperatures within the experimental puddles for every full hour for all 14 iButtons used. We calculated mean temperature and temperature ranges (difference between highest and lowest temperature per time slot) per hour. We assumed that tadpoles would use cooler zones when the water temperature was above their thermal preference range (22.6–27.0 °C, Drakulić et al. 2020) and vice versa. To compare the detailed tadpoles` locations with respective temperatures and available temperature ranges, we divided the experimental puddles into warmer and cooler zones (Figs 1B, 1C), based on the preceding temperature measurements. Every full hour, we divided the available temperature range in the middle between maximum and minimum temperature and determined the number of tadpoles present in each temperature category (warmer and cooler zones). The number of tadpoles in warmer zones was compared with those in the cooler zones at different times (0–23 h, N = 18) and different temperatures employing a Wilcoxon rank-sum test. To compare tadpole distribution at different temperatures, the data was sorted by minimum temperature and grouped in steps of up to 2.5 °C (Appendix I, N = 14–38, tests contained repeated measurements of the same tadpoles in cases where the water had the same temperature repeatedly during a trial). Moreover, we calculated the correlation (Spearman rank test) between the number of tadpoles and temperature. However, these analyses did not reveal if tadpoles move to preferred temperatures, due to a variable temperature distribution (e.g., deeper water was mostly warmer during decreasing temperature and cooler when temperature increased, Figs 1B, 1C, 4).

To investigate whether tadpoles move to preferred temperatures (Fig. 1D), we compared the spatial distribution of tadpoles with the hourly spatial temperature distribution. Temperature was measured in three spatially distinct zones: shallow water (using two iButtons at a depth of 2 cm), deep water (two iButtons at a depth of 14 cm), and surface water (two iButtons just below the surface above the deepest point). Temperature differences between these zones were analysed each full hour using Wilcoxon signed-rank tests (N = 18). Every hour, we recorded the number of tadpoles in three spatially distinct zones: shallow water (tadpoles assigned to the four iButtons at 2 cm and 6 cm depths), deep water (tadpoles assigned to the four iButtons at 10 cm and 14 cm depths), the surface water (tadpoles assigned to the six iButtons just below the water surface). Then we analysed changes in the mean tadpole distribution. We used Wilcoxon signed-rank tests to determine whether the number of tadpoles differed in these zones at times when a zone was expected to be preferred versus when it was expected to be avoided. Statistical analyses were conducted using R software (versions 4.2.2 and 4.3.3), and data was visualized using the ggplot2 package.

Finally, we determined if the tadpoles prefer shaded areas, which is particularly important for thermoregulation. The mean number of tadpoles and the mean tadpole density (tadpoles/m^2) in the shade and sun were calculated from noon to 4 pm (when shade occupied an area of maximal 30% of the container surface).

Results

Water temperatures in the experimental puddles

Overall temperatures ranged from 11.5 to 36 °C in the two experimental puddles. The highest mean temperature (of all 14 iButtons per trial and hour, N = 18; Fig. 2) was recorded at about 3 and 4 pm with an average of 30.5 °C; the lowest mean temperature we observed at 7 am with an average of 15.0 °C. On average mean water temperature was above the thermal preference range of *B. variegata* tadpoles (22.6–27.0 °C according to Drakulić et al. 2020) from 2 pm to 7 pm (≥ 27.5 °C) and below that preference range from 11 pm to 11 am (≤ 21.5 °C). The temperature ranges varied, and we observed the largest ranges between 11 am and 5 pm (mostly 2–4 °C), with a peak at noon and 1 pm (Fig. 2; difference between min. and max. temp, mean = 1.5 °C, median = 1.5 °C).

Thermal conditions at tadpoles' positions

Tadpoles were observed at temperatures below 20 °C in 44% and at temperatures above 30 °C in only 7% of all observations. The number of tadpoles was significantly higher in cooler zones from 10 am to 4 pm and higher in warmer zones from 6 pm to 7 am (Fig. 3, Appendix I, N = 18). At times when the temperature was on average outside their thermal preference range, the tadpoles mostly used zones with more suitable temperatures. However, in some cases, the results did not align with or contradicted our predictions. In all these cases, we could either observe that temperature distribution just changed (9/10 am, 5/6 pm, Fig. 4), the mean temperature was near the thermal preference range of the tadpoles (11 am, 6/7 pm, Fig. 2), the overall temperature ranges varied narrowly (8/9 am, Fig. 2), or some tadpoles moved towards the warmer shallow water (10/11 am, see below, Fig. 4). The results were presumably partly caused via the variable temperature distribution because more tadpoles were observed in the cooler or warmer zones when the deep water had this temperature and at

8/9 am and 5 pm the tadpole distribution changed simultaneously with the temperature distribution (Fig. 3, 4). Tadpoles were significantly more abundant in warmer zones at temperatures < 22.5 °C (and at 23.5/24.0 °C) and more abundant in the cooler zones at temperatures > 27.5 °C (Fig. 2, Appendix I). The differences became more pronounced when the temperature deviated further from the thermal preference of Yellow-bellied Toad tadpoles. Overall, the number of tadpoles was highly correlated with temperature, positive for tadpoles in cooler zones and negative for the tadpoles in warmer zones (Spearman rank test: r_s = (-) 0.33, p < 0.0001, N = 432 (24 measurements per group). When the available temperature in the experimental puddles was above or below the tadpoles' thermal preference range, they were primarily observed in puddle zones that linked better to their preferences.

Changes in spatial distribution of temperature and tadpoles

To investigate if tadpoles move towards zones with preferred temperatures, we analysed the spatial temperature distribution and compared it with the spatial tadpole distribution and their thermal preference. The spatial temperature distribution (Fig. 4, Appendix I) changed during the day. Shallow and deep water differed on average by 1.0 °C (median = 1.0 °C, N = 18). From 9 am to 5 pm, the temperatures were higher in shallow water but from 6 pm to 8 am, the temperatures were higher in deeper and surface water. The differences were significant throughout the day, except when temperature distribution changed at 5 pm (and 8 am). Surface and deeper water differed on average slightly by about 0.5 °C (median = 0.0 °C, N = 18). These

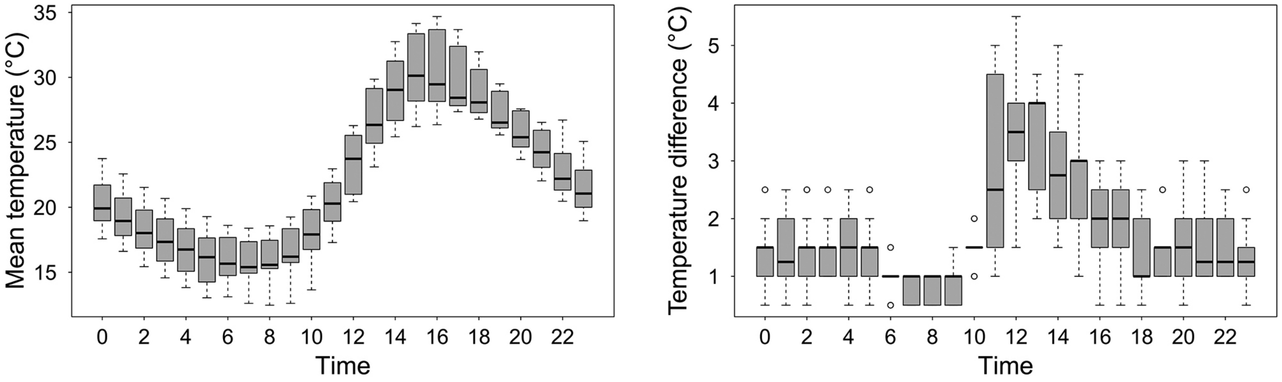

Figure 2. The experimental puddles underwent major natural fluctuations in temperature (left), but provided limited choice, minor temperature differences at any given time for behavioural thermoregulation (right). The figure shows mean temperatures and temperature ranges (i.e., the difference between minimum and maximum temperatures per time slot) in the experimental puddles over 24 hours, based on 18 trials conducted between July 22 and August 15, 2022.

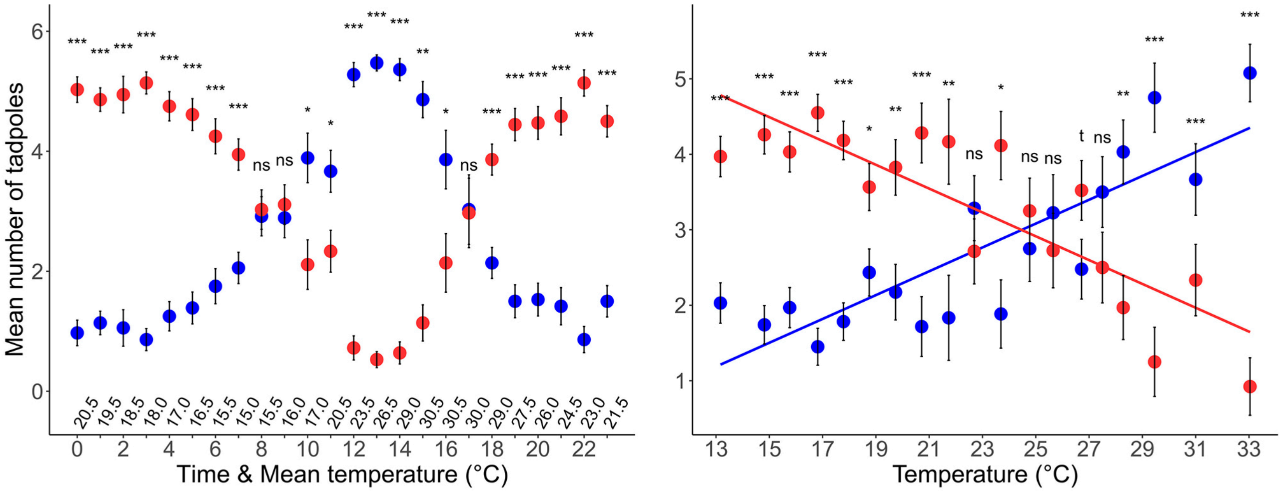

Figure 3. Yellow-bellied Toad (*Bombina variegata*) tadpoles predominantly used zones that were closely aligned with their thermal preferences. The figure shows the mean distribution of tadpoles between warmer (red) and cooler (blue) zones over 24 hours, based on 18 trials. The data is grouped by time (left, N = 18) and by temperature intervals in steps of up to 2.5 °C (right, N = 14–38 with n = 6–18 tested groups). Data points are shown with standard errors and trend lines. Significance codes for Wilcoxon rank-sum tests: ns (not significant) p ≥ 0.1, t (trend) p < 0.1, * p < 0.05, ** p < 0.01, *** p < 0.001. Further results are summarized in Appendix I.

differences were however significant from 10 am to noon and 3 pm to 6 pm when surface water was warmer (Appendix I).

Below we describe changes in the mean number of tadpoles across the three spatially distinct water zones (Fig. 4, N = 18). Tadpoles were consistently more abundant in the deep water compared to the shallow and surface water, except at 10 am. From noon to 5 pm, the mean in the deep water was higher (4 out of 6 tadpoles) than during most other times of day (3 tadpoles). In the shallow water, the mean peaked at 10 am and 11 am (more than 2 tadpoles) but dropped to low levels between 2 pm and 4 pm (less than 1 tadpole). In the surface water, the mean was mostly higher from 8 pm to 11 am (2 tadpoles) than between noon and 7 pm (1 tadpole).

We also tested if tadpole number differed in zones during times when a zone was expected to be preferred versus avoided (Fig. 5, Wilcoxon signed-rank tests, N = 18). In the shallow zone, the number of tadpoles should be higher from 9 am to 11 am, but lower from 2 pm to 4 pm and from 11 pm to 7 am. Tadpoles should be more numerous in the deep zone from 2 pm to 4 pm, and potentially from 11 pm to 7 am, and less numerous from 9 am to 11 am. Tadpoles should be less abundant in the surface zone from 3 pm to 4 pm and maybe more from 11 pm to 7 am. Tadpole numbers matched these assumptions, either significantly or by a trend to significance, except in the deep zone from 11 pm to 7 am versus 9 am to 11 am. In this case, it may not match because the surface zone was also preferable and used from 11 pm to 7 am.

In summary, the number of tadpoles was higher in the cooler deep zone in the warm afternoon and increased in the warmer shallow zone in the cool forenoon. Tadpole numbers were lower in the surface zone and in the shallow zone during the afternoon.

Shade preference

Shade preference was analysed due to its strong effect on thermoregulation. We analysed it from noon to 4 pm when shade covered no more than 30% of the container surfaces. In the shade, the number of tadpoles (on average 4–6 of 6 tadpoles, N = 7–12, Table 1) and hence the tadpole density was much higher than in the sun. At 2 pm and 3 pm shade was rare, water temperature was high, and tadpoles' preference for shade became very obvious because tadpoles accumulated in the few shaded areas i.e., at 2 pm (32.5 °C), 4 out of 6 tadpoles were seen in only 3% of the surface area; hence, the tadpole density was more than 80 times higher than in the sun (N = 7, Table 1).

Discussion

We analysed thermal relationships in the habitat use of Yellow-bellied Toad (*Bombina variegata*) tadpoles which develop in temporary puddles exposed to the sun, an extreme thermal environment. Our experimental puddles

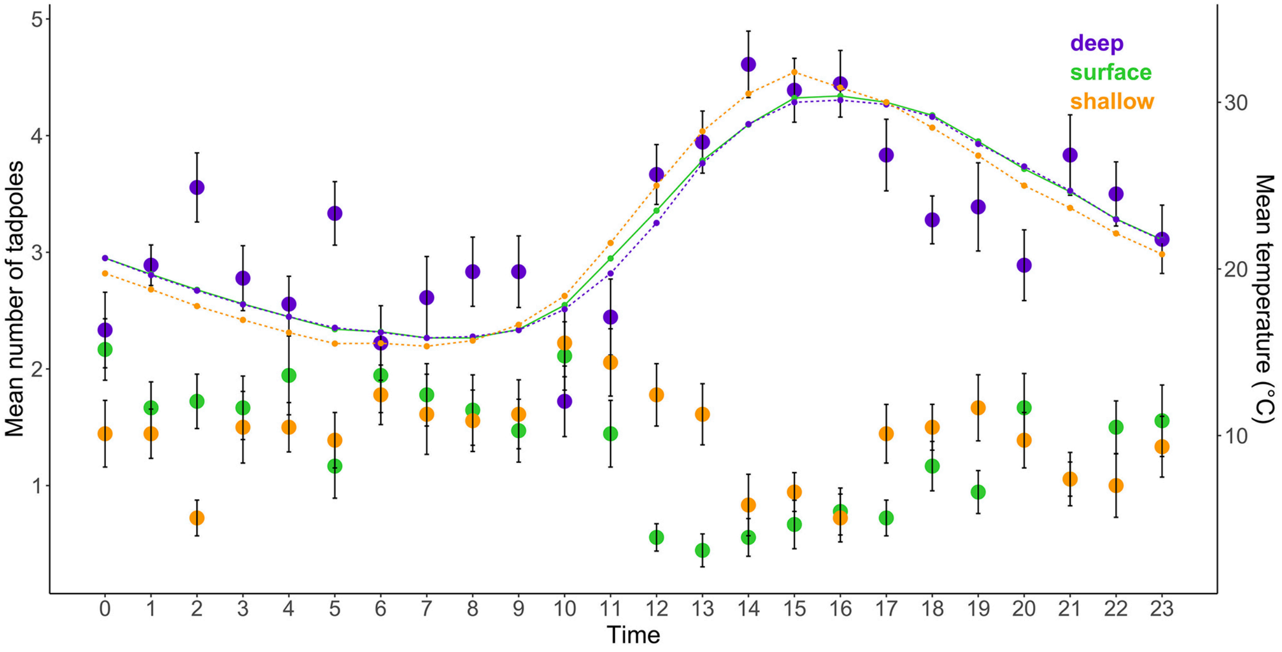

Figure 4. Yellow-bellied Toad (*Bombina variegata*) tadpoles used different water zones at various times of the day, mostly following their thermal preference. Based on 18 trials the figure illustrates changes in the spatial tadpole distribution (large points, with standard errors) and spatial temperature distribution (lines) over 24 hours, in the shallow zone (orange, 2–8 cm), deep zone (purple, 8–14 cm), and surface zone (green). Test results of temperature differences are provided in Appendix I.

Table 1. Yellow-bellied Toad (*Bombina variegata*) tadpoles accumulated in small, shaded areas during warmer afternoon hours. The table presented the time, mean percentage of shade coverage, mean number and density (per m²) of tadpoles in shaded and sunny areas, mean water temperature and sample size. Data was collected between 22 July and 15 August 2022.

Time	Shade coverage (in %)	Tadpoles in shade (number & density)		Tadpoles in sun (number & density)		Temp. (°C)	N
12	30	5.5	63	0.5	2	24.0	12
13	20	4.3	74	1.7	7	29.0	10
14	3	4.4	504	1.6	6	32.5	7
15	6	4.6	263	1.4	5	33.5	7
16	20	5.4	93	0.6	3	33.0	7

were subject to high temperature fluctuation and provided limited choice for thermoregulation, thus mirroring natural conditions. Spatio-temporarily tadpoles mostly used zones which were closer to their known thermal preference. Many moved to zones with preferable temperatures, indicating active thermoregulation, despite small differences in temperature. The tadpoles actively accumulated in the shaded areas during the warm afternoon.

The water temperature in our experimental puddles varied widely, ranging from 11.5 to 36.0 °C reflecting the conditions found in natural breeding sites, where temperatures can range from 12 to 40 °C (Drakulić et al. 2020). Small ponds are prone to high fluctuation in temperature because of their small volume of water. Shallow puddles exposed to the sun follow air temperature more closely with low minimum and high maximum temperatures, compared to deeper and larger waters less exposed to the sun (Nadeau et al. 2022). Natural water temperatures were often beyond the thermal preference range of *B. variegata* tadpoles, measured in laboratory experiments (22.6 to 27.0 °C according to Drakulić et al. 2020, and 24.6 to 29.7 °C according to Rühmekorf 1958b). Hence tadpoles should – when possible – compensate behaviourally for both higher and lower temperatures. The extent of potential thermal compensation depends on the temperature range, which in our study varied significantly within the puddles, although often extending over only a small range (mean = 1.5 °C). Small aquatic environments generally offer limited opportunities for thermoregulation due to their thermal homogeneity (Seebacher & Franklin 2005). However, temporary ponds are habitats of short duration that impact the evolution of amphibians (Butterworth et al. 2022). Even small differences in temperature may have significant physiological effects on the development of *B. variegata* tadpoles (Dittrich et al. 2016) and thus should influence habitat use. In the afternoon, when the tadpoles most likely face heat stress, temperature differences in our experimental puddles often ranged from 2 to 4 °C, thus, offering notable choices to avoid heat exposure that can reduce growth and survival of tadpoles (Ujszegi 2022). In our experiment, tadpoles mainly used zones aligning better with their thermal preference during periods when the water temperature was on average too warm or too cool. They increasingly used zones closer to their preferred range, when available temperatures deviated most from their preferred range. When temperatures matched the tadpoles' thermal preference, and thermoregulation thus was unnecessary, tadpoles were more evenly distributed.

The temperature distribution in our experimental puddles was highly predictable based on the time of day. The changes in the spatial tadpole distribution almost fitted the respective spatial temperature distribution indicating

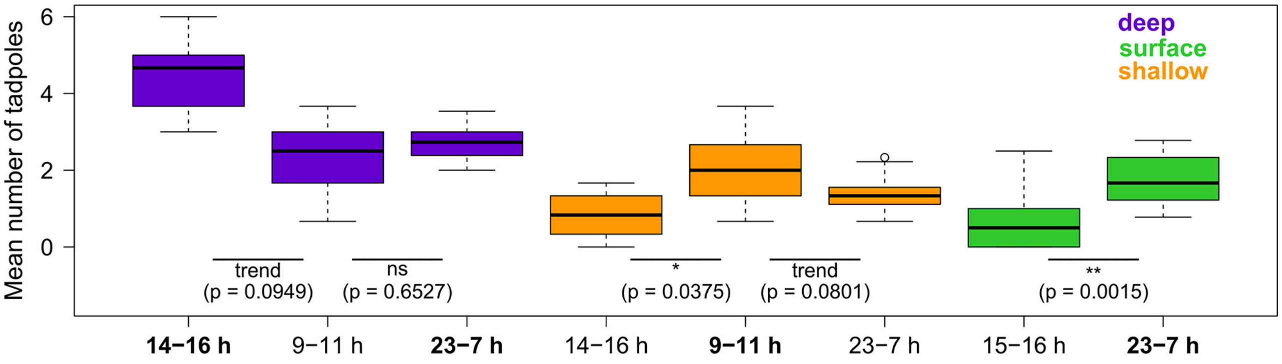

Figure 5. Yellow-bellied toad (*Bombina variegata*) tadpoles used different water zones at other times of the day, mostly following their thermal preference. Boxplots represent the mean number of tadpoles in the deep (purple), shallow (orange), and surface (green) zones during specific periods. The boxplots are labelled in bold for periods when tadpoles were expected to prefer a particular zone, and in regular font for periods when they were expected to avoid it. Test results of Wilcoxon signed rank tests (N = 18).

active thermoregulation and thermotaxis. The number of tadpoles was higher in the warmer shallow zone in the cool forenoon and the cooler deep zone during the afternoon. Fewer tadpoles remained at the water surface and shallow zone in the afternoon, likely due to higher temperatures and sun exposure. This pattern is similar to previous field studies i.e., in a 38 cm deep water body, inhabited by tadpoles of *Amerana boylii,* shallow water zones were warmer at forenoon and midday whereas deeper water zones were warmer in the evening. Tadpoles aggregated in shallow and warmer water in the forenoon, were found in deeper and cooler water around midday, equally distributed in the afternoon, and aggregated in the deep and warmer water in the evening (Brattstrom 1962). Tadpoles of the widespread American Toad *Anaxyrus americanus* moved to shallow waters when these warmed up and were present in the upper 1–2 °C of temperature range around noon. The tadpoles left shallow waters during late afternoon and were scattered during the evening and night (Beiswenger 1977). *Buergeria japonica* tadpoles stayed in cooler water zones when temperatures were high (Wu & Kam 2005). In fish, thermoregulatory movements occurred vertically, horizontally to cool waters, and occasionally to warm waters (Amat-Trigo et al. 2023).

We found that in the early afternoon, water temperatures were high, shaded areas were minimal and the tadpoles aggregated in the small shaded areas, likely to mitigate heat stress. Likewise, *Anaxyrus americanus* tadpoles move to particular microhabitats because of heat and light (Beiswenger 1977). Shade reduces exposure to harmful UVB radiation. However, tadpoles from four other amphibian species showed no evidence of UVB avoidance under natural conditions (e.g., Bancroft et al. 2008). Shade preference may also decrease predation risk by reducing the contrast with the environment i.e., *Argenteohyla siemersi pederseni* tadpoles tended to remain in darker or vegetated zones, possibly better suited for camouflage (Kehr et al. 2014). Low light intensity has been shown to enhance growth and development rates in some tadpoles (e.g., Kehr et al. 2014). Under natural conditions, not only temperature and light influence tadpole behaviour. Other factors, such as water body micro-structures, mud, food level, predators, individual health of tadpoles, oxygen saturation and other water chemistry, may also interact. Although field observations can provide valuable insights into thermal preferences of tadpoles, numerous variables aforementioned impact the tadpole response to temperature (Lucas & Reynold 1967).

Naturally, the highest temperatures occur in the afternoon in shallow sun-exposed water, which was avoided by the Yellow-bellied Toad tadpoles studied herein. Microclimates must be considered in order to understand the impacts of climate change. Average maximum temperature differences may reach up to 9.9–11.6 °C in aquatic microclimates located less than a meter apart (Nadeau et al. 2022), which can help species avoid their upper thermal limit. Thermal limits in anuran tadpoles are plastic and may depend on previously experienced temperatures; e.g. *Bufo lentiginosus* tadpoles that experienced high temperatures can shortly survive 43 °C (Davenport & Castle 1895). The plasticity of thermal tolerance is often unknown (Gunderson & Stillmann 2015) and can lead to an underestimated acclimatization potential. For instance, in *Rana uenoi* tadpoles developing in small ephemeral ponds, variations in the upper thermal limit were greater than generally assumed (Kim et al. 2022). However, the potential for adaptive responses in thermal limits to effectively ensure the survival of most species is limited (Bennett et al. 2021).

Conclusion

We herein report on the thermoregulatory behaviour of amphibian larvae in small thermally very challenging habitats. We observed that under semi-natural conditions the tadpoles used zones that fitted closer to thermal preferences, which were known from experimental studies. This was done although the temperature range was partly very small.

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Appendix I

Results of statistical analysis. Comparison of the number of Yellow-bellied Toad (*Bombina variegata*) tadpoles in warmer zones with the number of tadpoles in cooler zones at different times (N = 18) and different temperatures (N = 14–38 with n = 6–18 different groups per test) with Wilcoxon rank-sum tests (WRS). Comparison of temperatures between deep, shallow and surface zones at different times (N = 18) with Wilcoxon signed-rank tests (WSR).

Warmer vs. cooler at different times		Warmer vs. cooler at different temperatures				WSR: Temperature differences between shallow, deep and surface zone			
Time	WRS	Temp. (°C)	N	n	WRS	Time	Shallow vs. deep	Shallow vs. surface	Deep vs. surface
0	W = 0 p < 0.0001	11.5–14	34	6	W = 224 p < 0.0001	0	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 22.5 p = 1
1	W = 0 p < 0.0001	14.5–15	27	13	W = 74.5 p < 0.0001	1	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 30 p = 0.4386
2	W = 14.5 p < 0.0001	15.5–16	32	15	W = 173.5 p < 0.0001	2	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 20 p = 0.4054
3	W = 0 p < 0.0001	16.5–17	30	15	W = 63.5 p < 0.0001	3	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 32.5 p = 0.5637
4	W = 2 p < 0.0001	17.5–18	31	18	W = 151.5 p < 0.0001	4	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 36 p = 0.7762
5	W = 8 p < 0.0001	18.5–19	38	18	W = 490 p = 0.0153	5	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 48 p = 0.1628
6	W = 31 p < 0.0001	19.5–20	23	15	W = 138 p = 0.0053	6	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 15 p = 0.3173
7	W = 39.5 p < 0.0001	20.5–21	23	16	W = 98 p = 0.0002	7	V = 3 p = 0.0003	V = 0 p = 0.0002	V = 52.5 p = 1
8	W = 137.5 p = 0.4322	21.5–22	15	11	W = 50 p = 0.0084	8	V = 27.5 p = 0.0332	V = 24.5 p = 0.0711	V = 65 p = 0.1336
9	W = 146.5 p = 0.6211	22.5–23	21	14	W = 254 p = 0.3957	9	V = 105 p = 0.0097	V = 71.5 p = 0.0083	V = 30 p = 0.4386
10	W = 238 p = 0.0155	23.5–24	13	10	W = 35.5 p = 0.0104	10	V = 168.5 p = 0.0003	V = 153 p = 0.0003	V = 24.5 p = 0.0408
11	W = 225 p = 0.0446	24.5–25	24	15	W = 253 p = 0.4665	11	V = 153 p = 0.0003	V = 120 p = 0.0006	V = 0 p = 0.0014
12	W = 323.5 p < 0.0001	25.5–26	20	13	W = 229 p = 0.4271	12	V = 171 p = 0.0002	V = 171 p = 0.0002	V = 3 p = 0.0005
13	W = 324 p < 0.0001	26.5–27	23	15	W = 184 p = 0.0756	13	V = 171 p = 0.0002	V = 171 p = 0.0002	V = 38 p = 0.2068
14	W = 324 p < 0.0001	27.5	19	13	W = 244 p = 0.2337	14	V = 171 p = 0.0002	V = 171 p = 0.0002	V = 0 p = 0.0002
15	W = 307 p < 0.0001	28–28.5	16	12	W = 207 p = 0.0027	15	V = 170 p = 0.0002	V = 170 p = 0.0002	V = 0 p = 0.0006
16	W = 241 p = 0.0114	29–30	12	8	W = 131.5 p = 0.0006	16	V = 143 p = 0.0118	V = 138 p = 0.0215	V = 4.5 p = 0.0020
17	W = 163.5 p = 0.9613	30.5–32	18	9	W = 30.5 p < 0.0001	17	V = 100.5 p = 0.5121	V = 84.5 p = 0.9651	V = 12 p = 0.0119
18	W = 38 p < 0.0001	32.5–34	13	7	W = 165 p < 0.0001	18	V = 0 p = 0.0003	V = 0 p = 0.0002	V = 0 p = 0.0114
19	W = 14 p < 0.0001					19	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 10.5 p = 0.2714
20	W = 15 p < 0.0001					20	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 44.5 p = 0.0757
21	W = 19 p < 0.0001					21	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 50.5 p = 0.3341
22	W = 0.5 p < 0.0001					22	V = 0 p = 0.0003	V = 0 p = 0.0002	V = 16 p = 0.4170
23	W = 8 p < 0.0001					23	V = 0 p = 0.0002	V = 0 p = 0.0002	V = 21 p = 0.8514