

## Thermoconformity even in hot small temporary water bodies: a case study in yellow-bellied toad (*Bombina v. variegata*)

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**Abstract.** The small sized yellow-bellied toad inhabits small temporary water bodies. These puddles are used by this species in both open landscapes and closed forested habitats. The thermal characteristics of these puddles in the open and closed habitat types showed considerable difference. In laboratory conditions the toad selected a narrow and low preferred temperature range (20.5-23.3°C). The measured voluntary thermal maximum (VTMax<sub>water</sub>) and critical thermal maximum (CTMax) was  $35.9 \pm 1.3^\circ\text{C}$ , and  $38.3 \pm 0.5^\circ\text{C}$  respectively. In the field, the toads using the surface water layer were thermoconformers irrespective of the actual thermal environment. The high VTMax<sub>water</sub> and CTMax presumably permit the use of such a thermoregulatory strategy.

**Key words:** Amphibian, small temporary water bodies, thermoconformity, voluntary and critical thermal maximum.

### Introduction

Ectotherms use external sources of heat to alter their body temperature, for instance, they can absorb heat from the solar radiation or utilize heat from the substrate via conduction (Spellerberg 1976, Carey 1978, Brattstrom 1979, Avery 1982, Huey 1982, Hutchinson & Dupré 1992, Zug 1993). Many reptiles can cope with variable thermal environments and maintain optimal body temperature by means of behavioural thermoregulation (Grant & Dunham 1988, 1990, Whithers 1992, Kearney & Predavec 2000, Grbac & Bauwens 2001, López et al. 2002, Gvoždík 2002, Herczeg et al 2003). The main options for behavioural thermoregulation are adjusting seasonal and daily activities to the environment's thermal opportunities and thermal microhabitat selection during activity. The two extreme behavioural thermoregulatory strategies are thermoconformity (no behavioural regulation) and active thermoregulation (Huey & Slatkin 1976). Huey and Slatkin's (1976) cost-benefit model of lizard thermoregulation predicts variation in the appropriate strategy with respect to the costs (time and energy devoted, increased quality of the environment).

The majority of amphibians has difficulties in behavioral thermoregulation, due to the water evaporation across permeable skin, and also because the fact that heat gained by exposure to radiation is usually lost by evaporation of water (Carey 1978, Brattstrom 1979, Pough *et al.* 1983, Tracy et al. 1993, Moore & Sievert 2001, Shoemaker et al. 1992). The integument of most amphibians lacks an effective resistance to evaporative water loss, thus the necessity of a moist skin and its evaporative water/heat loss limit the control of body temperature. An amphibian is generally characterized by activity body temperatures at the ambient temperature or below it (Zug 1993, Whithers 1992).

In some cases adult amphibians can respond behaviorally to temperature changes, e.g. some typical responses involve avoidance of extreme temperatures, shifts in activity patterns, and thermoregulation (Brattstrom 1979, Hutchinson & Dupré 1992, Navas 1997). In highlands amphibians try to avoid low temperatures (Carey 1978) and in tropical habitats amphibians react by specific behavior to avoid high temperatures (Navas 1996, Navas 1997). The substrate selection is another thermoregulatory behaviour of frogs and salamanders in tropical highlands (Feders 1982, Sinsch 1989, Navas 1996). Even basking is a common thermoregulatory behavior in some anuran genera (Brattstorm 1979, Bradford 1984, Hutchinson & Dupré 1992), but it is more effective in larger species and those with reduced cutaneous water loss (Tracy et al. 1993). Tropical arboreal tree-frogs apply physiological control of evaporative cooling to stabilize their body temperature when environmental temperature is high (Pough *et al.* 1983). These frogs allow their body temperature to track the environment's temperature until their body reaches 38-40°C, then their skin glands begin secretion, thus evaporation allows the frog maintain a stable body temperatures even if the ambient temperature reaches 44-45°C (Zug 1993). In *Rana temporaria*, the tadpole aggregations may have a pronounced thermoregulatory function too (Surova 2006). During cloudy days the temperature inside

aggregations is higher compared to ambient ones, but in sunny days vice versa. The permanent moves of tadpoles up and down can create convective streams, which can significantly change the water temperature.

The yellow-bellied toad (*Bombina v. variegata* Linnaeus 1758) is widespread in Europe as well as in Romania (Cogălniceanu et al. 2000). It usually lives in small temporary water bodies in a great variety of habitats mostly at medium and high elevations (Fuhn 1960). There is important difference between the thermal characteristics of the water bodies situated on open landscapes, such as pastures and meadows and puddles situated on closed, forested habitats. During the warm months the water and air temperature of the water bodies on the open landscapes can reach very high temperatures, which can be dangerous for the toads, in contrast with the forested habitats, where the environmental temperatures are much lower. In the late spring and in the summer months the toads are breeding in all habitat types, without preference to a warmer or colder type, but there is a possible acclimatization to these different environments. If the toad behaviorally responds to these two thermally different habitat types, there must be a different relationship between the animal and the ambient temperature in the two cases.

In this study, the thermal relations between the yellow-bellied toad body core and its natural environment was focused on and respectively the determination in laboratory conditions of the preferred temperature range, the voluntary thermal maximum, temperature border, which constraints the toad to leave the water body, and the critical thermal maximum, which represent the minimum degree of the temperature range, which can compromise the life of the toad.

## Material and methods

### *Study species and site*

The yellow-bellied toad is member of the ancient Discoglossidae family. It is a mostly diurnal small sized toad (less than 50 mm). Its representative character is the bright yellow belly with blackish round shaped spots (Cogălniceanu et al. 2000). Its reproductive season can reach sometimes even into the autumn months (Sos, unpublished data).

I studied a population of yellow-bellied toad near Rupea locality (Braşov county; 46°02'-25°13', average altitude 451m; figure 1). The study area represents the contact zone between the Transylvanian Basin and the East Carpathians, respectively the Olt and Târnava Valley. The average annual air temperature is 9,5°C, and the average maximum and minimum air temperatures are 30°C, respectively -30°C (Anonymus 1993). I included individuals from habitats separated in two categories: an open (which were situated in open landscapes like pastures, meadows, grasslands) and a closed (which were situated in forested habitats) type. The average difference in the air-, and water temperatures between the open and forested habitat types were 8.5°C, respectively 7.4°C, respectively.

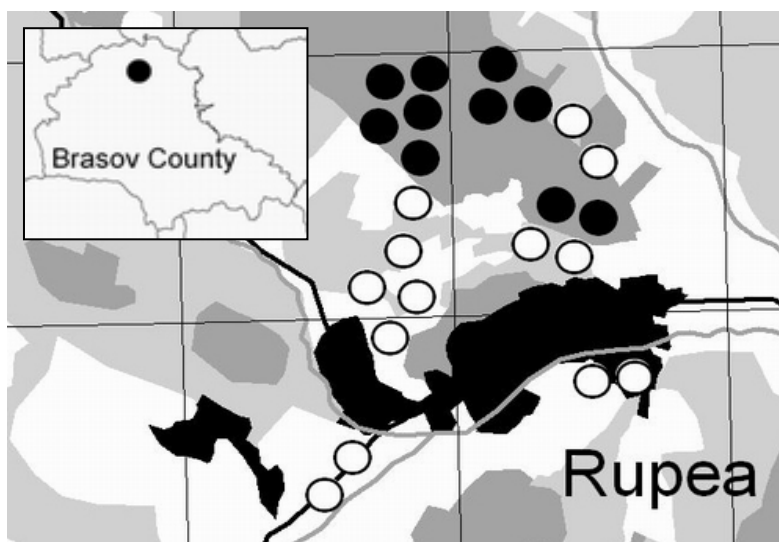
### *Measurement of thermal preference, voluntary and critical thermal maxima*

Hertz. et al. (1993) proposed a research protocol that enables the testing of explicit hypotheses about behavioral temperature regulation in field-active ectotherms. By using this protocol, the distribution of field measurements of body temperatures ( $T_b$ ) are compared to expected or null distribution of body temperatures that would result if the animal behaved randomly with respect to its thermal environment (Heath 1964, Grant & Dunham 1988, Hertz 1992a,b, Kearney & Predavec 2000). Series of indices are proposed that quantify how the distributions of body temperatures and randomly sampled operative temperatures differ with respect to the set-point range (Hertz et al. 1993, Kearney & Predavec 2000, Blouin-Demers & Weatherhead 2001). Set-point ranges ( $T_{set}$ ) are best estimated by selected body temperatures in the laboratory, on in an environment that is independent of the ecological costs and constraints that can influence temperature (Hertz et al. 1993).

I used a laboratory aquatic thermal gradient (Gvoždík 2003) to estimate the  $T_{set}$ . For this experiment 10 adult toads were captured in 16.-31.05.2006 time period from different habitat types. The body length of tested toads exceeded the 30 mm snout-vent length (SVL) in all cases. Experimental animals were housed in plastic cages filled with water before the experiments. I used a plastic tube of 1 m length 105 mm in diameter. The tank was longitudinally divided into 5 200 mm long, partially separated compartments of equal size, with different water temperatures. The tank was filled with water to a depth of 5 cm. Before the experiment the tank showed a horizontal thermal gradient from 14°C to 30°C. For determination of temperature preferences, toads were placed individually into the central compartment of the gradient, 12 h before the experiment, for habituation to the gradient environment. The cloacal body temperatures of toads were recorded every 30 minutes starting from 08.00 to 20.00 hour. To estimate  $T_{set}$  for each individual, I used the limit value of central 80% of the selected temperature (Bauwens et al. 1996; Grbac & Bauwens 2001; Gvoždík 2002).

The voluntary thermal maximum (VTMax: Cowles & Bogert 1944, Pough & Gans 1982) represents the  $T_b$  at which, or above which the animal moves away from conditions that would further raise its temperature (Cowles & Bogert 1944, Spellerberg 1976, Kearney & Predavec 2000). In this study the VTMax was determined as the water temperature, at which the toad accelerated its swimming rhythm more than twice of the normal rhythm time and decreased the pause between the swimming periods attempting to escape from the chamber.

The critical thermal maximum (CTMax) is frequently considered as the temperature, at which animals lose a self-righting response (Navas 1997). The temperature for onset of muscular spasms, used in this study, may be a more appropriate parameter to measure, because it is less variable than the righting response decrement (Hutchinson 1987, Witz 2001). The former can give lower estimates, but is mostly harmless for the animals. For each trial I recorded the body and water (CTMax<sub>water</sub>) temperature at which the toad began to spasm (Witz 2001). When spasm observed, I removed the toad from the chamber and immersed it in a tank with water at temperature closed to the ambient air temperature. This procedure was successful in returning all toads to sub-lethal  $T_b$  quickly.



**Figure 1.** The study area near Rupea (Braşov County, Romania). Light grey areas represents the pastures and meadows, and the dark grey areas represent the forested (beech) areas. The white and black spots represent the studied pools from the open and closed habitats, respectively.

For measuring the VTMax<sub>water</sub> and CTMax I used a plastic tank filled with water. The plastic tank was placed in a steel tank filled with water, which was placed on the heating pad. Between the two tanks a wood isolation prevented the plastic tank from melting. The toads were individually placed in the plastic container and the water was slowly heated. After each experiment the water in each tank was changed. For each toad the VTMax<sub>water</sub>, CTMax and the maximum water temperature (CTMax<sub>water</sub>) were measured in same experiment.

For this experiments five male, five female and five subadult toads were captured in May 2006 on the open landscapes and another 15 on the forested habitat. The sex, the mass to the nearest 0.01 g and the SVL to the nearest 1.0 mm with a ruler also were recorded. For testing the SVL and the mass effect on tested temperature, I separated the toads in two SVL (1: < 3.9cm, 2: > 4.0cm) and in two mass (1: < 3.9g, 2: > 4.0cm) groups.

#### *Measurements of body temperatures and operative temperatures in the field*

I conducted field studies between 13 May and 01 June in 2006. For collecting data I selected days with 25-30°C average shaded air temperature (measured in open landscapes) and I chose the period between 11-17 hours daytime, when the experienced temperature is the highest.

I captured the toads with a net with 150 mm diameter. I recorded the cloacal temperature with a digital thermometer inserted not deeper than 5 mm in the toad's cloaca. After checking the sex and measuring the SVL and mass of the toads, I released them in the original puddle. Besides  $T_b$ , I measured shaded air temperature ( $T_{air}$ ) at 1 m above the water surface and water temperature ( $T_{water}$ ) in 50 mm deep in the water at the site of each capture. I also measured the operative temperature ( $T_e$ ), an estimate of body temperature of an animal that does not thermoregulate and had reached thermal equilibrium; e.g. Bakken et al. 1985, Bakken 1992, Hertz et al. 1993) available for each individual (see below).

The expected or null distribution of available  $T_b$  is estimated by randomly sampling  $T_e$  from the animal's habitat. After each toad's  $T_b$  record, I used four models to estimate the available operative temperature ( $T_{e1-e4}$ ). Because of high conductivity of water and small thermal differences between spots in the same water layer, I supposed these four measurements will be enough for estimating the available temperature in the water surface layer that the toads are usually using. The physical models were constructed from hollow pipes (Shines & Kearney 2001). The model had 40 mm length, 20 mm width and was 10 mm in height. The pipes were filled with rubber silicon and painted in light grey approaching the original color of yellow-bellied toad which lives in open landscapes. In the studied closed habitats the toads had darker color. I chose the lighter grey colored model

because in open landscapes the color of the ectotherms is thermally important, while in closed habitats it has no importance (Zug 1993). For the thermometer in one end of the models an entrance with 5 mm length was burred, and in the opposite end the models were fixed to a steel wire. In the field the wire was picked in the soil layer and the models were positioned in the surface layer of water, close (15-20 cm) to the place where the toad was captured. In this case the surface size and the contact with the substrate are obviously more important factors which affect the model temperature than the color and the orientation (Shine & Kearney 2001). After 10-15 minutes the temperature of models was recorded.

To map the available temperatures in different layer of small temporary water bodies, I measured two different pool types (with 50 mm and respectively 100 mm deep water bodies) in open and closed habitats at two days with different daily average shaded air temperature (07.06.2006: 27°C and 09.07.2006: 33°C; temperatures were measured between 11-17 hour). The water temperature was measured at each 50 mm deep in the middle and at the margin of the pond starting from the surface layer (figure 2).

### Evaluating the extent of temperature regulation

Thermoregulation implies that toads would distribute (both spatially and temporally) in a non-random fashion in their thermal environment, such that  $T_b$  values deviate less from the preferred temperature range, than do  $T_e$  values (Hertz et al. 1993, Kearney & Predavec 2000). The accuracy of thermoregulation is tested with measuring the mean of the absolute values of deviations ( $\bar{d}_b$ ) (in degrees Celsius) of  $T_b$  from  $T_{set}$ . If  $T_b < T_{set}$ ,  $d_b = T_b - T_{set}$  (lower border), if  $T_b > T_{set}$ ,  $d_b = T_b - T_{set}$  (upper border) and if  $T_b$  falls in  $T_{set}$ ,  $d_b = 0$ . The lower value of  $\bar{d}_b$  show a greater accuracy of temperature regulation, i.e., the less  $T_b$  deviates from  $T_{set}$  (Hertz et al. 1993, Kearney & Predavec 2000). The quality of the thermal environment from the organism's perspective is tested with measuring  $\bar{d}_e$  indices, which is computed as  $\bar{d}_b$ , but for the  $T_e$  values. The higher quality of the thermal environment is proven by a lower value of  $\bar{d}_e$ , i.e. the less  $T_e$  deviates from  $T_{set}$  (Hertz et al. 1993, Kearney & Predavec 2000). The observation that  $\bar{d}_b \geq \bar{d}_e$  would suggest that animals are not regulating. The  $\bar{d}_b < \bar{d}_e$  relation prove a behavioral thermoregulation.

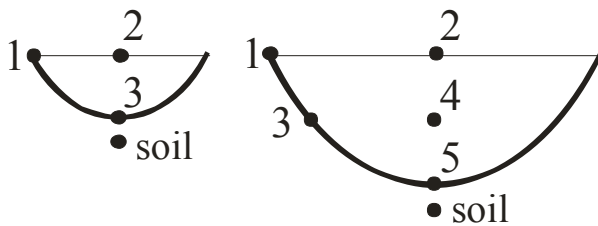


Figure 2. The spotted layers in the 50 mm and in the 100 mm deep temporary water bodies (for details, see text).

The effectiveness of temperature regulation is measured from the following formula:  $E = 1 - (\bar{d}_b / \bar{d}_e)$  (Hertz et al. 1993).  $E$  approach to zero, when the magnitudes of  $\bar{d}_b$  and  $\bar{d}_e$  is similar. In this case the effectiveness of thermoregulation is low, because the  $T_b$  deviates from the  $T_{set}$  in similar way as the  $T_e$ .  $E$  approaches one and thus effectiveness of thermoregulation is increasing, when  $T_b$  deviates less than  $T_e$  from the  $T_{set}$ . The  $T_{e1-4}$  dates were used in four measurement (the 1-4 groups together), which can characterize four possible thermal relations. Blouin-Demers & Weatherhead (2001) proposed other indices to test the effectiveness of thermoregulation to account for the potential bias of Hertz et al 's (1993) index that include a ratio. The  $\bar{d}_e - \bar{d}_b$  formula (Blouin-Demers & Weatherhead 2001) should be used to quantify the extent of departure from perfect thermoconformity. This index provides an open-ended scale where negative numbers represent animals that avoid thermally favorable environment, zero represent perfect thermoconformity, and positive numbers represents animals that thermoregulate with some extant. In the present study the  $\bar{d}_e$ ,  $E$  and  $\bar{d}_e - \bar{d}_b$  was calculated for each model temperature separately ( $T_{e1-4}$ ).

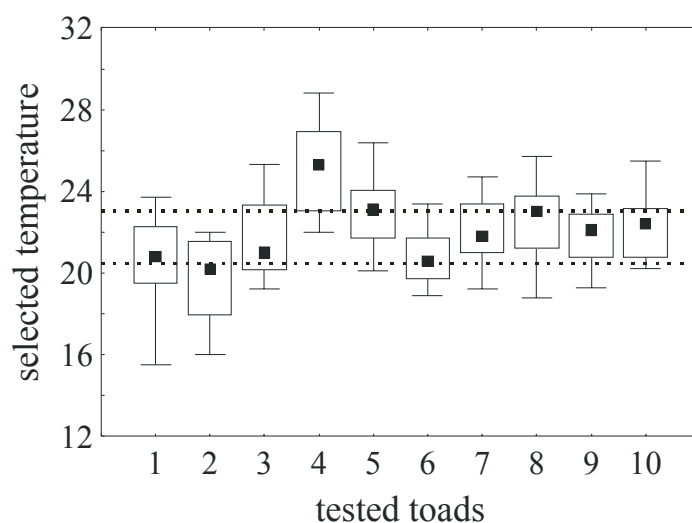
All statistical analyses were performed with Statistica Version 0.6 using  $P < 0.05$  as a standard criterion of statistical significance. All data sets were examined to ensure that they met the assumptions of the specific test prior to analysis.

## Results

### Set-point range, voluntary and critical thermal maximum

The overall lower and upper set-point temperatures ( $T_{set}$ ) were  $20.5 \pm 1.3^\circ\text{C}$  and  $23.3 \pm 1.5^\circ\text{C}$  (mean  $\pm$  1 SE). This surprisingly narrow preferred temperature range is partly explained by the preference of the toads for the second compartment counted from the warmer end. One of the toads selected a higher temperature range (number 4, see figure 3), but the rest preferred more and less a similar range.

The voluntary thermal maximum (VTMax<sub>water</sub>) significantly differed between the sexes, the females began their escape activity in warmer temperature (Kruskal-Wallis U test,  $H_{[2,27]} = 7.45$ ,  $P < 0.01$ ). The VTMax<sub>water</sub> of the two body length category did not differ (Mann-Whitney U test,  $z = -1.5$ ,  $P > 0.5$ ), but the heavier toads started their escape activity later than the lighter toads ( $z = -2.0$ ,  $P < 0.01$ ), however these differences were smaller than 1°C degree (heavy:  $36.3 \pm 1.3^\circ\text{C}$  vs. light:  $35.4 \pm 1.3^\circ\text{C}$ ), furthermore I used the average temperature (i.e.,  $35.9 \pm 1.3^\circ\text{C}$ ). No significant difference was found between the critical thermal maximum (CTMax) in sexes (Kruskal-Wallis U test,  $H_{[2,27]} = 4.59$ ,  $P > 0.05$ ). The two SVL and mass category showed a statistically significant response: the longer and heavier toads (the two categories correspond in that case) resisted to higher temperatures (Mann-Whitney U test,  $z = -2.25$ ,  $P < 0.05$ ). The average difference was lower than 0.5°C. Furthermore I used the mean temperatures (i.e.  $38.3 \pm 0.5^\circ\text{C}$ ). The critical maximum water temperature (CTMax<sub>water</sub>) not differed between sexes (Kruskal-Wallis U test,  $H_{[2,27]} = 0.77$ ,  $P > 0.5$ ), SVL categories (Mann-Whitney U test,  $z = -0.85$ ,  $P > 0.1$ ) or mass categories ( $z = -0.85$ ,  $P > 0.1$ ). The measured CTMax<sub>water</sub> is  $40.3 \pm 0.2^\circ\text{C}$ . The VTMax<sub>water</sub>, CTMax and CTMax<sub>water</sub> is similar in the toads from the open and closed habitats (Mann-Whitney U test,  $z = -0.02$ ,  $P > 0.5$ ,  $z = -1.11$ ,  $P > 0.1$ , respectively,  $z = -0.38$ ,  $P > 0.5$ ).



**Figure 3.** The individually, preferred body temperatures (plot: median, box: 20% and 80% percentile, whiskers: min-max) of the tested *B. variegata* exemplars. Superimposed on the figures is the set-point range (dashed lines).

### ***Body and operative temperatures. The quality of the thermal environment and the extent of temperature regulation***

There were no differences in SVL (1 way ANOVA,  $F_{[1,141]} = 0.53$ ,  $P > 0.1$ ) or in mass ( $F_{[1,141]} = 1.38$ ,  $P > 0.1$ ) between sexes. The categories made on basis of SVL showed that in the smaller animal the two parameters are highly correlated ( $B = -1.01$ ,  $R^2 = 0.38$ ,  $F_{[1,140]} = 12.73$ ,  $P < 0.0005$ ), but in the larger toads exists a stagnation in SVL, while the mass is increasing ( $B = 1.63$ ,  $R^2 = 0.60$ ,  $F_{[1,168]} = 14.23$ ,  $P < 0.0001$ ). The toads tested from the open and closed habitats showed a significant difference in SVL (one-way ANOVA,  $F_{[1,165]} = 9.33$ ,  $P < 0.01$ , open:  $3.82 \pm 0.07$ ,  $N = 96$  vs. closed:  $4.17 \pm 0.08$ ,  $N = 71$ ) and body mass ( $F_{[1,165]} = 14.01$ ,  $P < 0.001$ , open:  $4.17 \pm 0.2$  vs. closed:  $5.6 \pm 0.2$ ). However the ANOVA indicated that from the factors SVL and habitat type only the habitat type ( $P < 0.0005$ ) has an influence on temperature (table 1).

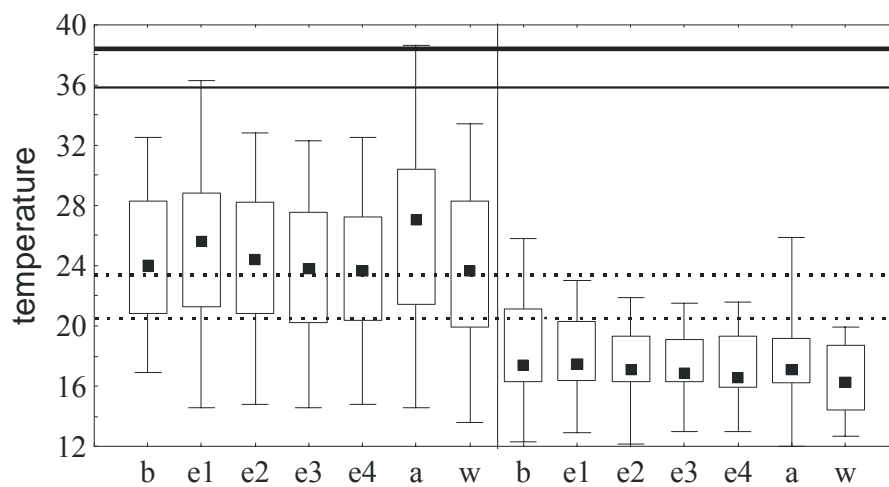
The model temperatures differed from each other in two cases: in opened habitat type the  $T_{e1}$  showed higher temperature than  $T_{e3}$  and  $T_{e4}$  (Mann-Whitney U tests,  $P < 0.05$ ), the rest were similar ( $P > 0.1$ ). The mean deviations of models temperatures from  $T_{set}$  ( $\bar{d}_{e1-4}$ ) not differed significantly from each other in the two different habitat type ( $P > 0.1$ ).

The differences between the measured body temperature of the toads and the model temperatures indicate cautions in handling of models. The disturbed surface water layer can rapidly change its

temperature influenced by the deeper colder water. This was obvious mainly in open habitat measurements where the relations between  $T_b$  and  $T_{e1-4}$  differed significantly. There  $T_b$  was significantly smaller than  $T_{e1}$  (Wilcoxon paired test,  $P < 0.05$ ; figure 4), similar with  $T_{e2}$  ( $P > 0.05$ ) and higher than the  $T_{e3}, T_{e4}$  ( $P < 0.05$ ), even if the  $T_{e1-4}$  temperatures can accurately explain the change of  $T_b$  (Spearman Rank Order Correlations,  $r_{b-e1} = 0.84, r_{b-e2} = 0.86, r_{b-e3} = 0.89, r_{b-e4} = 0.88$ ). So the first measurement of model ( $T_{e1}$ ) was higher, the second ( $T_{e2}$ ) was similar and the third and fourth ( $T_{e3-4}$ ) were smaller than  $T_b$ , thus we can follow the descending temperature of models. The measurement of the mean deviations of different temperatures from  $T_{set}$  ( $\bar{d}$ ) is not so sensitive to this temperature change. In open habitat the mean deviation of body temperatures from  $T_{set}$  ( $\bar{d}_b$ ) was higher than the  $\bar{d}_{e1}$  ( $P < 0.001$ ; table 1) and was similar to  $\bar{d}_{e2}, \bar{d}_{e3}, \bar{d}_{e4}$  ( $P > 0.05$ ). Broadly, in open habitats the toads experience same temperatures like the models.

**Table 1.** Yellow-bellied toad body temperatures ( $T_b$ ), operative temperatures at random position in surface water layer ( $T_{e1-4}$ ), the shaded air ( $T_{air}$ ) and water ( $T_{water}$ ) temperatures in open ( $N = 96$ ) and closed ( $N = 71$ ) habitats, respectively the mean deviations ( $\bar{d}_x$ ) of measured temperatures from the set point range.

Habitat	Open	Closed
$T_b$	24.4 ± 0.3	18.1 ± 0.3
$T_{e1}$	25.1 ± 0.4	17.9 ± 0.2
$T_{e2}$	24.2 ± 0.4	17.5 ± 0.2
$T_{e3}$	23.8 ± 0.4	17.4 ± 0.2
$T_{e4}$	23.7 ± 0.3	17.4 ± 0.2
$T_{air}$	26.6 ± 0.5	18.1 ± 0.3
$T_{water}$	21.5 ± 0.2	16.4 ± 0.4
$\bar{d}_b$	2.44 ± 0.25	2.85 ± 0.26
$\bar{d}_{e1}$	2.99 ± 0.29	2.87 ± 0.22
$\bar{d}_{e2}$	2.59 ± 0.25	3.11 ± 0.23
$\bar{d}_{e3}$	2.43 ± 0.24	3.14 ± 0.22
$\bar{d}_{e4}$	2.29 ± 0.23	3.23 ± 0.22
$\bar{d}_{air}$	4.64 ± 0.37	3.38 ± 0.18
$\bar{d}_{water}$	2.88 ± 0.30	4.08 ± 0.25



**Figure 4.** Field cloacal temperature of *B. variegata* (b), operative or model (e1-4), shaded air (a) and water (w) temperature from open (left) and closed (right) habitat types. Superimposed on the figures is the set-point range (dashed lines), the voluntary thermal maximum (thin line) and critical thermal maximum (solid line).

In open habitats the  $T_b$  was significantly higher than the  $T_{\text{water}}$  (Wilcoxon paired test,  $P < 0.05$ ; figure 4) and smaller than  $T_{\text{air}}$  ( $P < 0.05$ ) and was more influenced by  $T_{\text{water}}$  (Spearman Rank Order Correlations,  $r = 0.78$ ), than by  $T_{\text{air}}$  ( $r = 0.66$ ). The mean deviation of body temperatures from  $T_{\text{set}}$  ( $\bar{d}_b$ ) was higher than the  $\bar{d}_{\text{air}}$  and  $\bar{d}_{\text{water}}$  ( $P < 0.001$ ). Obviously the toad body temperature is influenced by the water and air temperature together. The higher influence of water temperature in body temperature change can be interpreted like a tendency of the toads to avoiding the warmer air temperature, when they select the colder water, but it can be just the influence of semi aquatic environment.

In closed, forested habitats the  $T_b$  was significantly higher than  $T_{e2}$ ,  $T_{e3}$ ,  $T_{e4}$  (Wilcoxon paired test,  $P < 0.05$ ; figure 4) and was similar with  $T_{e1}$  ( $P > 0.05$ ). The correlations between  $T_b$  and  $T_{e1-4}$  were weaker comparative with open habitats (Spearman Rank Order Correlations,  $r_{b-e1} = 0.75$ ,  $r_{b-e2} = 0.70$ ,  $r_{b-e3} = 0.68$ ,  $r_{b-e4} = 0.64$ ). There the  $\bar{d}_b$  was similar with all the deviations ( $P < 0.0005$ ; table 1). The  $T_b$  was significantly higher than  $T_{\text{water}}$  ( $P < 0.05$ ; figure 4) and was similar with  $T_{\text{air}}$  ( $P > 0.05$ ), and was similarly influenced by  $T_{\text{water}}$  ( $r = 0.56$ ) and  $T_{\text{air}}$  ( $r = 0.55$ ). There the  $\bar{d}_b$  was similar with  $\bar{d}_{\text{air}}$  ( $P < 0.0005$ ; table 1), but higher than the  $\bar{d}_{\text{water}}$  ( $P > 0.5$ ). There the toads experience a higher body temperature than the models and the 5 cm deep water layer, but was similar with the air temperature. This relation can be interpreted like a tendency to more terrestrial activity of toads to avoid the colder water environment, but the distance of body temperature and models temperature dates from the preferred body temperature range did not show any accuracy of thermoregulation.

The difference in the mean deviations compared between the two habitat types is smaller in  $\bar{d}_b$  (Mann-Whitney U test,  $P > 0.1$ ) than in the other mean deviations ( $\bar{d}_{e1}$ :  $P > 0.5$ ,  $\bar{d}_{e2}$ :  $P > 0.05$ ,  $\bar{d}_{e3}$ :  $P < 0.01$ ,  $\bar{d}_{e4}$ :  $P < 0.0005$ ;  $\bar{d}_{\text{air}}$ :  $P > 0.05$ ,  $\bar{d}_{\text{water}}$ :  $P < 0.0005$ ). There is a tendency for higher deviations in closed habitats, but for  $\bar{d}_{\text{air}}$  which is higher in open habitat. The higher similarity in  $\bar{d}_b$  between the two habitats with different thermal environment types can be interpreted as a primitive control of body temperature.

The differences in spotted  $T_e$  resulted in different counted effectiveness (table 2). In all the habitat types the  $E$  indices of effectiveness of thermoregulation showed a tendency to thermoconformity. The measured higher model temperature revealed in one case ( $T_{e1}$ ; table 2) active thermoregulation, but this is probably a mistake in data collection. In open habitats the greater indices referred to the fact that air temperature results greater independence from the air temperature. The indices proposed by Blouin-Demers & Weatherhead 2001 ( $\bar{d}_e - \bar{d}_b$ ) showed the same result. In some cases the negative results indicate animals that avoid thermally favorable environment or are forced to be out of this.

**Table 2.** The effectiveness of thermoregulation in *B. variegata* estimated after the indices proposed by Hertz et al. 1993 ( $E$ ) and Blouin-Demers & Weatherhead 2001 ( $\bar{d}_{\dots} - \bar{d}_b$ ).

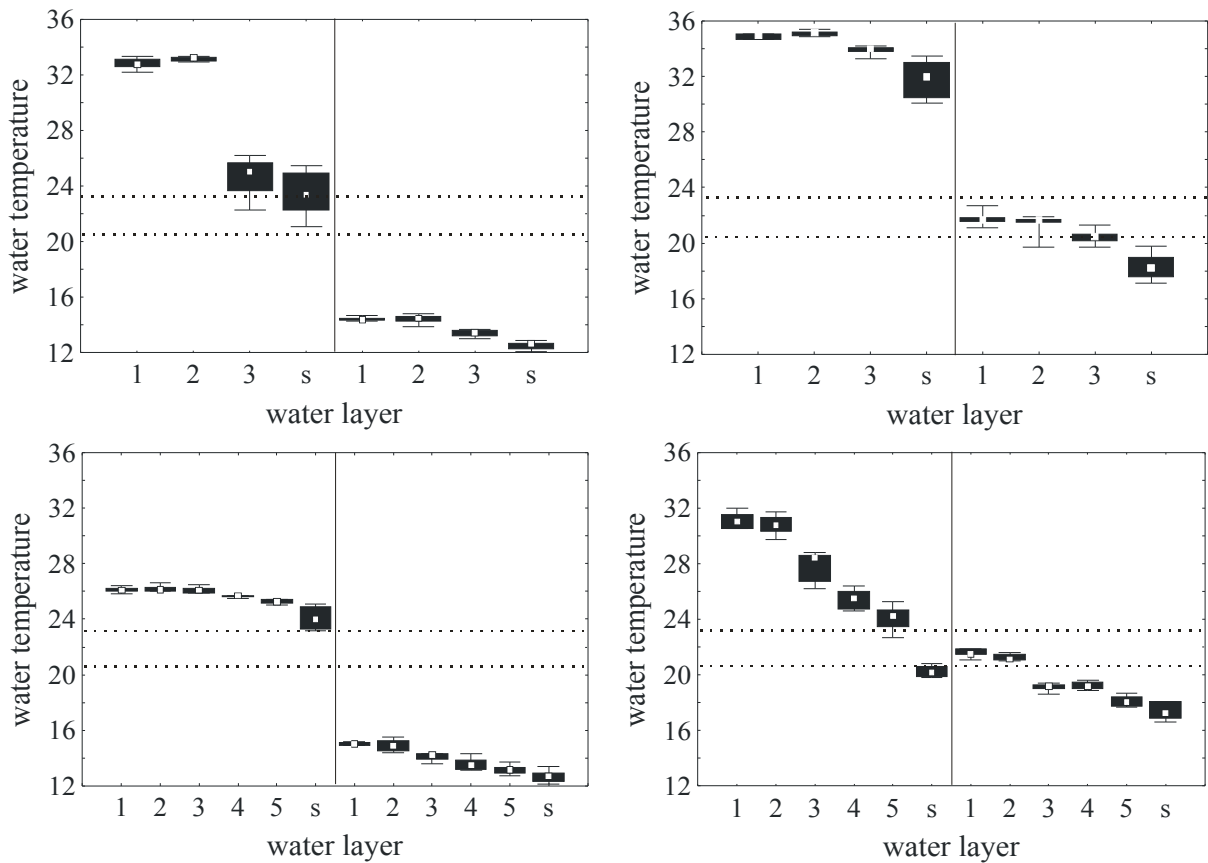
Sources	Open habitat		Closed habitat	
	$E$	$\bar{d}_{\dots} - \bar{d}_b$	$E$	$\bar{d}_{\dots} - \bar{d}_b$
$\bar{d}_b, \bar{d}_{e1}$	0,91 ± 0,01	0,55 ± 0,17	0,82 ± 0,01	0,01 ± 0,15
$\bar{d}_b, \bar{d}_{e2}$	0,31 ± 0,08	0,15 ± 0,13	0,26 ± 0,06	0,26 ± 0,17
$\bar{d}_b, \bar{d}_{e3}$	0,13 ± 0,13	-0,01 ± 0,14	0,27 ± 0,06	-0,28 ± 0,18
$\bar{d}_b, \bar{d}_{e4}$	0,27 ± 0,08	-0,15 ± 0,14	0,29 ± 0,05	0,38 ± 0,19
$\bar{d}_b, \bar{d}_{\text{air}}$	0,52 ± 0,05	2,20 ± 0,25	0,21 ± 0,07	0,52 ± 0,22
$\bar{d}_b, \bar{d}_{\text{water}}$	0,18 ± 0,16	0,43 ± 0,20	0,34 ± 0,05	1,22 ± 0,22

Between the water layers in the 50 mm and 100 mm deep temporary ponds in all cases (two dates, two habitat types) was a significant difference (Kruskal-Wallis test,  $P < 0.005$ ; figure 5). This means that a larger temperature range can be exploited. In open habitats the temperature of water layers exceeded in all de cases the toad's  $T_{\text{set}}$  just in the shallow puddles (e.g. 5 cm deep) in the warmer days (i.e.. at 33°C shaded air temperature), while in closed habitats the water layers temperature reach the toad's  $T_{\text{set}}$  just in the

warmer days (i.e. at 33°C shaded air temperature) without reference to the puddles size. The temperature of toad used surface water layer exceeds the upper border of  $T_{set}$  in all cases measured in open habitats. In closed habitats, in days with 27°C shaded air temperature the surface water layer is below the lower border of  $T_{set}$  but is situated in  $T_{set}$  in warmer days (33°C).

**Discussion**

The preferred temperature range in amphibians is expected to be wide and low, which is characteristic for thermoconformer ectotherms (Huey & Slatkin 1976). The measured surprisingly narrow preferred temperature range (20.5 - 23.3°C) in yellow-bellied toad is narrower and lower than in other temperate-zone amphibians (*Rana catesbiana*: 22-28°C, *R. temporaria* 23-30; Zug 1993), but the methods used to identify this range was different. The fact that amphibian do select body temperature is not a new fact (Carey 1978, Feders 1982, Sinsch 1989, Navas 1996, Navas 1997, Moore & Sievert 2001, Gvoždík 2003). Measures of locomotor performance such as swimming and jumping are depressed at low temperature (Navas et al. 1999). Acoustic signals and digestion rate are also strongly dependent on temperature (Denöel 1998). The effect of temperature on calling is important, mainly in frogs characterized by high calling efforts (Navas 1996b). In *B. v. scabra* the pulse rate, the note per minute, the call duration, the interval among the notes and the dominant frequency is strongly influenced by body temperature (Vasara et al. 1991), thus the tendency to select an optimal temperature range can be expected to a higher extent in male toads.



**Figure 5.** The temperature (plot: median, box: 20% and 80% percentile, whiskers: min-max) of different water layers in 50 mm deep (up) and 100 mm (down) temporary water bodies in two dates (left column: 07.06.2006 and right column: 09.07.2006).

The abbreviations (1-3, 1-5 and s) represent the spotted water and soil layers (see figure 2). The dates at left and at right from the vertical line were measured in open, and closed habitats, respectively. Superimposed on the figures is the set-point range (dashed lines).

The higher temperature resistance in females or in larger individuals is a coincidence in most cases, because the female yellow-bellied toads are generally heavier (Fuhn 1960). The slightly greater resistance to higher temperature can be explained by the surface area/body weight ratio: the larger body core with smaller surface area is more protected in case of warmer environment. This is true for *R. temporaria* too in mountainous region (Vences et al. 2002). Partly for that reason the frogs under 1 g are not capable to thermoregulate (Tracy et al. 1993).

In larger water bodies tropical highland aquatic frogs can experience higher and less fluctuating body temperatures than the sympatric terrestrial, nocturnal species (e.g. Andean frogs; Navas 1997). In our climate the larger water bodies can offer a lower and less fluctuated body temperature than in the small temporary water bodies. In the study area the toads related to the small temporary puddles are under the constraint of high water and air temperatures. In open habitats the toad can choose or is forced to higher body temperature by the warmer air temperature. This is an obvious effect of their lifestyle: they spend most of their time in the surface water layer. There the toad can try to disperse the effect of the warmer air temperature, using the colder water layers. In the closed habitat the toads rather preferred the warmer air temperature than the colder water, but there the toad can use both aquatic and terrestrial environments without caution to thermal characteristics. In this study the thermal relations between the toads and their microenvironments shows a slight tendency for avoiding the hotter air temperature in open habitats and for avoiding the colder water temperature in closed habitats.

In open habitats the toads can use different layers of water with strongly different temperature to optimize their temperature, similarly to the temperate-zone snakes under retreats (Huey et al. 1989) or amphibiae in soil layers (López et al. 1998). The thermal characteristics of water bodies from forested habitats are colder, thus not so generous (except the hottest days when the water temperature can reach the preferred optimum range), but are safer in warmer days. Here the behavioral and physiological performances are presumably lower values but the toads are not in the danger of overheating, as opposed to in open landscapes, where the water temperature can reach even the toad's critical thermal maximum. The toads captured in the surface water layer are more likely to be thermoconformers, with a body temperature similar to the used surface water layer like the majority of amphibians (Zug 1993).

But active behavioral thermoregulation could be otherwise relevant in the yellow-bellied toads. All tested toads were captured in water, but their prior terrestrial movements can profoundly change their body temperature. Even swimming in deeper layers can strongly influence their body temperature. During swimming the toads are not totally immersed: the upper surface of their heads is in direct contact with the air. This can be an important factor what can change its body temperatures. In the open habitats the more aquatic toads can experience a larger range of temperature s due to the differences between water and air temperature ranges. In closed habitats the toad can choose between a reasonable warm air temperature and the colder water temperature.

Even the lower voluntary thermal maximum of the yellow-bellied toad ( $35.9 \pm 1.3^{\circ}\text{C}$ ) except that critical thermal maximum ( $38.3 \pm 0.5^{\circ}\text{C}$ ) and critical maximum water temperature ( $40.3 \pm 0.2^{\circ}\text{C}$ ), is higher than the CTMax in highland tropical frogs like *Telmatobius culeus* ( $32.6^{\circ}\text{C}$ ; Hutchison 1982), *Eleutherodactylus bogotensis* ( $34.5^{\circ}\text{C}$ ; Hutchinson 1965) and *Colosthetus subpunctatus* ( $32.6^{\circ}\text{C}$ ; Hutchinson 1965). The CTMax is similar to that of *Hyla labialis* ( $38.5^{\circ}\text{C}$ ; Mahoney & Hutchinson 1969). The CTMax of high-elevation tropical frogs are similar with those of low-elevation tropical amphibians (Navas 1997). But in these tropical frogs the CTMax was estimated from the righting response lapse (Synder & Weathers 1975, Navas 1997), which is higher than the onset of muscular spasm method used in this study (Witz 2001). Thus, the yellow-bellied toad has a higher temperature tolerance than some tropical frogs.

The CTMax of yellow-bellied toad is closed to the CTMax of the Taiwanese heat tolerant tadpoles of *Buergeria japonica* inhabiting streams and geothermal hot springs, that is over  $41^{\circ}\text{C}$  (Wu & Kam 2005). These *B. japonica* tadpoles select ca.  $37^{\circ}\text{C}$  water temperatures even the thermal profile of the plots varied seasonally. In the warmer spring and summer tadpoles could be found only in the cooler sections of the pools. These results suggest that extreme heat tolerance and behavioral thermoregulation (i.e., heat avoidance behavior) are the main means by which *B. japonica* tadpoles cope with the high temperatures in geothermal hot springs (Wu & Kam 2005).

Consequently the thermoconformity of the yellow-bellied toad is not a direct attendant from its incapability to thermoregulate; it can be result of the toad adaptations to the hot temporary water bodies. Similar to the *B. japonica* tadpoles its high heat tolerance and possible behavioral thermoregulation permit it to occupy this hot environment. When the water reaches these dangerous temperature levels for the toads,

they possibly avoid the higher temperature, by leaving the exposed hot temporary puddles, sometimes selecting a colder puddle in a forested area (Sos, unpublished data). The high air temperature can be an important factor which precipitates time of habitat change. In this hot environment the humidity level is obviously less important.

The seasonality of the yellow-bellied toad's thermal physiology, and the active body temperature regulation of toads in their terrestrial environment need to be addressed in the future.

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