# Staying warm or moist? Operative temperature and thermal preferences of common frogs (*Rana temporaria*), and effects on locomotion

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Ambient temperature largely determines the body temperature of amphibians, and thus their hydration state and physiological performance. Microhabitat conditions chosen by terrestrial amphibians may represent a trade-off between high ambient temperatures, which maximize performance but cause high rates of water loss, and low temperatures, which, in turn, slow desiccation, but potentially hinder performance. We determined the operative temperature of common frogs (*Rana temporaria*) by placing 3% agar models in different microhabitats and measuring their temperature and water loss. Temperature measurements derived from the models accurately matched the body temperature of live frogs placed in the same microhabitat. Operative temperatures were lower than ambient temperatures on a warm day, probably because of evaporative water loss, but they were similar to or even slightly higher than ambient temperatures on a cool day, possibly because of warmth from the substrate. Frogs in the field selected moist and cool habitats, and their body temperatures ranged from 15 to 21 °C. In a temperature gradient in the laboratory, captive frogs chose significantly higher temperatures (19.4±1.7 °C) when the gradient floor was covered entirely with wet sand than when sand was wet in the cool end, but dry in the warm end (17.6±2.5 °C). The relevance of the preferred temperature was assessed through jumping performance experiments, using frogs with different body temperatures. Jump length was lower at low body temperature (6 °C) than at higher body temperatures, and peaked at 15 °C. Our results suggest that the frogs select favourable microhabitats of intermediate temperature, which could result in reduced water loss and peak physiological and behavioural performance.

Key words: agar models, body temperature, jumping performance, microhabitat, skin temperature, thermal gradient

# INTRODUCTION

**B**ody temperature  $(T_b)$  of animals affects their physiological and behavioural performance. Unlike endotherms, ectothermic animals do not produce metabolic heat to defend a constant  $T_b$ ; instead, their  $T_b$ is largely determined by the temperature of their environment (Tracy, 1975; Navas et al., 2008). However, ectotherms are known to thermoregulate behaviourally by selecting favourable microhabitats (Lillywhite, 1970; Christian & Weavers, 1996; Vences et al., 2002; Seebacher & Franklin, 2005). In doing so, they maintain a range of  $T_b$  in which physiological and behavioural performance are optimized (Walvoord, 2003; Seebacher & Franklin, 2005).

Warm microhabitats are favourable for ectotherms, as locomotor performance is reduced at low ambient temperature ( $T_a$ ), and improves with increasing temperature (Rome et al., 1992). Locomotion is crucial for survival and fitness as it facilitates escape from predators, foraging and detection of mates. In amphibians, locomotor performance generally increases with increasing  $T_{\rm b}$ , until it reaches a performance plateau and decreases rapidly at very high temperatures (Wells, 2007). The optimal temperature range varies between species, with some showing wide performance plateaus over a 10–20 °C temperature range, while others have narrower thermal optima (Tracy, 1979; Hirano & Rome, 1984; Knowles & Weigl, 1990; Walvoord, 2003).

Although they are advantageous for locomotion, high ambient temperatures result in higher evaporative water loss (EWL) in terrestrial amphibians. The wet skin of most amphibians offers essentially no barrier to water loss through evaporation (Young et al., 2005). The selection of microhabitats has been found to be influenced by the hydration state of amphibians, thus indirectly affecting thermoregulation (O'Connor & Tracy, 1992; Tracy & Christian, 2005). Amphibians should prefer warm and moist microhabitats to regulate their hydration state and maximize physiological performance. However, a higher  $T_a$  is often linked to dry conditions, so amphibians may face a trade-off between choosing a lower  $T_a$  to avoid

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desiccation and a higher  $T_{\rm a}$  that maximizes locomotor performance through increased  $T_{\rm b}$ .

 $T_{\rm b}$  and water loss of ectotherms in different microhabitats can be simulated by placing physical models of similar size, shape and absorptivity into the natural habitat of a particular species (e.g. Bartelt & Peterson, 2005; Tracy et al., 2007; for a review see Dzialowski, 2005). Such models produce a meaningful thermal index, termed environmental or operative temperature ( $T_{\rm e}$ ).  $T_{\rm e}$  is defined as the  $T_{\rm b}$  of an animal in thermal equilibrium with its environment (Bakken et al., 1985; Dzialowski, 2005). Models made out of 3% agar imitate evaporative properties of amphibian skin well (Spotila & Berman, 1976; Navas & Araujo, 2000) and have been used in a variety of studies of the thermal biology and water loss of amphibians (Navas, 1996; Schwarzkopf & Alford, 1996; Navas & Araujo, 2000).

The aim of this study was to explore the thermal ecology of common frogs (Rana temporaria) by determining  $T_{\rm o}$  under different conditions and comparing those temperatures to thermal preferences in the field and under captive conditions. We also investigated the effect of  $T_{a}$ on the physical performance of the frogs. We first placed different-sized physical models (simulating immature and fully grown frogs) in various microhabitats and measured their temperature and water loss to determine  $T_{e}$  in R. tem*poraria*. We predicted that  $T_e$  would be similar for frogs of different sizes in the same habitat. The accuracy of our temperature measurements was tested by placing models and live specimens in the same microhabitat, predicting that their  $T_{\rm b}$  would be similar. Second, while we made measurements of  $T_{e}$ , we caught frogs in different microhabitats and determined  $T_{\rm b}$  and environmental parameters. Third, we investigated the thermal preferences of captive frogs in a temperature gradient. We hypothesized that the frogs would select moist microhabitats where their  $T_{\rm b}$  would not exceed 25 °C and water loss is minimized. Fourth, to provide a performance context to the temperature selection data, i.e. to show the consequences of being at different  $T_{\rm a}$ , we investigated the effect of  $T_{\rm b}$  on locomotor activity of R. temporaria. We measured jump lengths of frogs with different  $T_{\rm b}$  and hypothesized that jumping performance would increase with  $T_{\rm b}$  and would be optimal near the average  $T_{a}$  of their microhabitat.

# MATERIALS AND METHODS

#### Study animals and validation of agar models

We caught 50 common frogs (*Rana temporaria*) of different body sizes in the Tuchola Forests region, Poland (53°57'N, 17°48'E). Frogs were found in pine forests (*Pinus sylvestris*), where the floor was patchily covered mainly by sphagnum moss and blueberry bushes, and in open grassland close to Lake Brzeźno and small streams feeding the lake. They were weighed to  $\pm 0.01$  g (Scout Pro SP 402, Ohaus Corp., Pine Brook, NJ, USA), and their snout–vent length (SVL), maximum head width and maximum body width were recorded (Table 1). These measurements were used to carve three different sizes of physical models: the size of the smallest and largest live specimen caught, and an average size deriving from the

**Table 1.** Body measurements of 50 *Rana temporaria* (mean  $\pm$  SD and range) that were used to carve different sized physical models. SVL = snout-vent length. Body mass of the frogs is also presented.

		Head	Body	
	SVL	width	width	Body
	(cm)	(cm)	(cm)	mass (g)
Mean $\pm$ SD	4.15	1.39	1.87	5.49
	$\pm 0.96$	$\pm 0.32$	±0.56	$\pm 3.90$
Minimum	2.70	0.80	1.20	1.32
Maximum	7.00	2.40	3.30	18.08

mean of each measurement from all individuals. Models were cut out from blocks of 3% agar, which simulates the thermal and evaporative properties of live frogs (Navas & Araujo, 2000).

To determine the accuracy of measurements using the physical models, we compared  $T_{\rm b}$  of the models to that of live specimens. Medium-sized models and newly caught frogs were placed in a plastic container (which provided protection from wind) and placed in the sun, while a second pair was placed in a mesh cage in the shade nearby. The pairs remained in these microhabitats for 30 min, after which internal temperature was recorded by inserting a type K thermocouple (associated with a digital thermocouple reader; ZyTemp TN40ALC, Radiant Innovation Inc., HsinChu, Taiwan) into the cloaca of the frog and 3 mm under the dorsal surface of the model. We also measured the following environmental variables after the 30 min experimental period:  $T_a$  at 2 cm above the ground, relative humidity (RH; Vaisala HMI41 humidity and temperature indicator with a HMP44L probe, Vaisala Oyi, Helsinki, Finland) and wind speed (Kestrel 4000 pocket weather meter, Nielsen Kellerman, Boothwyn, Pennsylvania, USA). The experiment was repeated four times for each environment over the course of one day, using different models and frogs. The agar models accurately reflected frog  $T_{\rm b}$ , as the internal temperature of the physical models ( $T_{e \ sun}$ : 20.0±1.0 °C;  $T_{e \ shade}$ : 18.6±1.0 °C) and of live specimens ( $T_{b \ sun}$ : 21.2±0.9 °C;  $T_{b \ shade}$ : 18.7±0.8 °C; all values are means  $\pm$  SD) could not be distinguished statistically (ANCOVA:  $F_{1,13}$ =1.72, P=0.21) when placed in the same environment.  $T_b$  of both models and frogs was significantly lower in the shade than in the sun (ANCO-VA: *F*<sub>113</sub>=16.75, *P*<0.01).

To determine whether skin temperature  $(T_{skin})$  could be used as a surrogate for  $T_b$  in subsequent experiments, we measured  $T_{skin}$  and cloacal  $T_b$  of the 50 frogs caught initially with an infrared thermometer and a thermocouple (ZyTemp TN40ALC, Radiant Innovation Inc., HsinChu, Taiwan). The frogs were held by their hind legs and placed on the ground and  $T_{skin}$  and  $T_b$  were measured within a few seconds to minimize the warming effect of the hand (Navas & Araujo, 2000). Temperature measurements were made indoors, at a room temperature of 22 °C, after the frogs had been habituated to this temperature for 30 min. These conditions were not meant to represent any natural situation, but were used to test whether  $T_{\rm skin}$  and  $T_{\rm b}$  were equal.  $T_{\rm skin}$  varied between consecutive measurements with slight changes in the distance between thermometer and frog skin (length of the infrared beam), and was therefore not used as a surrogate for  $T_{\rm b}$ . We report  $T_{\rm b}$  for subsequent experiments, as it was a more precise temperature measurement.

Nineteen medium-sized frogs (SVL 4.14 $\pm$ 0.79 cm and body mass 7.39 $\pm$ 2.31 g; mean  $\pm$  SD) were kept in captivity for one week. Of these, 13 individuals were used for the locomotion experiment and six for temperature preference tests in a temperature gradient. We were not able to distinguish between sexes, since most individuals were immature and paired vocal sacs and the nuptial pads on the first finger of males, used for gripping females during mating, were not yet discernable. Frogs were kept in plastic containers containing moist moss and live insects caught at the field site were provided daily after experiments. All frogs were released at the site of capture following completion of the study.

## Measurements of $T_{e}$

 $T_{e}$  was estimated by placing medium-sized models in different microhabitats (3-4 replicates each) at the site of initial capture. Four microhabitat categories were distinguished: warm & dry (sun, dry soil, low humidity), warm & wet (sun, moist soil, high humidity), cool & dry (shade or under plant cover, dry soil, low humidity) and cool & wet (shade or under plant cover, moist soil, high humidity). Two replicates (models of the same size) were placed next to each other in a particular microhabitat for 30 min. We weighed all models before and after this experimental period to determine water loss. After the 30 min period,  $T_{\rm L}$ of the models was measured as described above. Simultaneously, we recorded  $T_a$  at 2 cm above the ground, wind speed and RH. All RH values obtained in this study were converted to vapour density using Smithsonian meteorological tables (List, 1966). We calculated vapour density of the air (VD<sub>air</sub>) of each microhabitat from the recorded RH and  $T_a$ , and vapour density at the frog surface (VD<sub>skin</sub>) from the ambient RH and  $T_{skin}$ . Vapour density deficit (VDD) is the difference between VD<sub>air</sub> and VD<sub>skin</sub>.

To test for differences in  $T_{e}$  between model sizes, we placed the three freshly made models of different sizes in an open spot (no plant cover, soil covered with dry moss) and three others in a shaded spot (next to large bush, grass cover, moist sandy soil), 3 m apart. Data were collected for one sunny, warm day (day 1) and one overcast, cool and windy day (day 2). On both days,  $T_{\rm h}$  of the models was recorded every 1 s, and averaged every 1 min, using copper-constantan (Type-T) thermocouples interfaced to a data logger (Campbell Scientific Inc. 21X Micrologger, Logan, Utah, USA). We recorded  $T_a$  at 2 cm above the ground in both environments using thermocouples, either manually every 1 h or continuously by connecting them to the data logger. RH and wind speed were recorded in both spots three times daily. Models were weighed every 2 h and were replaced when they had lost more than 15%

of their initial mass.

## $T_{\rm b}$ of wild frogs

To compare  $T_{\rm e}$  to temperatures selected by wild frogs, we sampled an area consisting of different habitat types, namely meadow (grass up to 80 cm high), pine forest and artificial garden (short grass and flower beds, next to buildings), in close proximity to a lake (<15 m) and further away from it (>15 m). All habitat types were sampled with equal intensity three times a day (0900–1800), on the same days the models were placed in the environment. We caught 29 wild frogs of various sizes over the course of the day and measured their  $T_{\rm b}$ . The environmental conditions ( $T_{\rm a}$  at 2 cm above the ground, RH and wind speed) were also recorded in each place a frog was captured.

## Temperature preference

In addition to  $T_{\rm b}$  selected in the field, we measured thermal preferences of six captive frogs in a thermal gradient system, consisting of a long and narrow aluminium trough  $(120 \times 10 \text{ cm})$ . One end of the trough was heated to 50 °C by a FBH 604 Fisherbrand® thermostat and the second end was cooled down to 0 °C by a FBH 635 Fisherbrand® cryostat, resulting in a temperature gradient ranging from 1 to 45 °C. The temperature gradient was divided into 16 compartments of equal length. Temperature increases were greater between the five compartments at each end of the gradient (2-4 °C difference between two neighbouring compartments) than in the six middle compartments (1.5-2 °C difference). Frog movements in the gradient were not restricted. However, in each experimental series, low cardboard barriers were placed at 12 °C and 40 °C in the gradient to ensure that frogs were choosing very low (<12 °C) or high (>40 °C) temperatures. A single frog was placed in the middle of the gradient and was allowed to habituate for 10 min. Thereafter, we observed its behaviour in the gradient for 1 h, during which the compartment the frog stayed in was recorded every 60 s. Substrate temperatures in each compartment were measured after the 1 h observation period using a thermocouple. The six captive frogs were tested in two experiments on consecutive days. In the first experiment, the floor of the gradient was covered entirely with wet sand. This experiment allowed us to assess general thermal preferences of the common frog when there were no constraints on hydration. In the second experiment, the gradient floor was covered with wet sand at the cold end (0-12 °C) and dry sand at the warm end (13-45 °C). These temperatures were chosen based on preferences found in the first series. The lowest temperature preferred by frogs on wet sand was 15 °C (see results below), but we put the wet sand at 3 °C cooler than the lowest preferred temperature to achieve a clear distinction between the regulation of  $T_{\rm b}$  or hydration.

#### The effect of $T_{\rm b}$ on locomotion

As an indicator of the consequences of choosing different  $T_{\rm b}$ , we tested jumping performance at three temperatures. We altered  $T_{\rm b}$  of 13 captive frogs by placing them into different  $T_{\rm a}$  or water temperatures. Three temperature categories were used: warm (sunny day, mean  $\pm$  SD  $T_{\rm a}$  = 22.25 $\pm$ 2.05 °C), medium (temperature of lake water:

12.25±2.19 °C) and cold (water with melted ice: 2 °C). The same individuals were exposed to each temperature category on consecutive days, always at the same time of day to minimize the effects of daily rhythms. They were habituated to a certain temperature for 10-15 min, or until  $T_{\rm b}$  had adjusted to the environmental temperature (<5 min in ice water). Cloacal  $T_{\rm b}$  was measured and the frog was immersed in very dilute water-soluble paint and placed in a jumping arena. This arena consisted of a wooden base  $(200 \times 50 \text{ cm})$ , on which paper was placed. A plastic mesh was used to cover the sides and top so that the frog could only jump in one direction. Frogs were stimulated to jump by gently tapping their hind legs. A plastic box containing moss was placed at the end of the jumping arena and the frogs jumped towards this hiding place. Immediately after the jumps,  $T_{\rm b}$  was measured again, but generally did not differ from the initial value. Environmental parameters  $(T_{a} \text{ at } 2 \text{ cm above the ground, RH and wind speed})$  inside the jumping arena were also recorded at the start and during the experiment.

The length of the individual jumps was later measured using the paint imprints. A jump was measured from the end of the hind legs of one imprint to the end of the hind legs of the next imprint. Depending on the length of the jumps and willingness to jump, we obtained four to 10 jumps per frog, and the mean of all jumps for each individual at a particular temperature was calculated. We calculated the temperature coefficients ( $Q_{10}$ ) from the relationship between mean jump length and  $T_b$ . We also measured SVL of the frogs to determine whether the maximum jump length of each individual was related to frog size.

#### Statistical analysis

All data were tested for normality (Kolmogorov–Smirnov and Shapiro–Wilk tests) and homogeneity of variance (Levene's test). Log, square-root or arcsine transformations were used when data were heteroscedastic. A General Linear Model (ANCOVA) was used to identify which environmental factors affect  $T_b$  of the wild frogs. The categorical predictor was body size and continuous predictors were  $T_a$ , wind speed,  $VD_{air}$  and  $VD_{skin}$ . Variables were removed from the analysis until the best model fit was found; the adjusted  $R^2$  value was used as an indicator for model fit. For each experimental series in the temperature gradient, the proportion of time spent in cold ( $\leq 12$ °C) and warm (>12 °C) areas were compared by paired *t*-test. Temperatures selected by the frogs (i.e. the  $T_{a}$  at which each frog spent most of the time) were compared between the series using a paired *t*-test. The temperatures frogs chose in each minute of observation in each experimental series were exposed to a Levene's test, assuming that the frogs would show greater variance in their temperature choices when they faced a trade-off (wet and dry sand). For the locomotion experiment,  $T_{\rm b}$  and mean and maximum jump lengths were compared between the three temperature categories using repeated-measures ANOVA. To eliminate a possible effect of body size on jumping performance, this analysis was performed on SVL-corrected jumping data. Post-hoc comparisons were conducted with Tukey's Honestly Significant Difference test for equal sample sizes, followed by a Bonferroni correction for multiple comparisons (Rice, 1989). Linear regression analysis (GLM) was used to test for a relationship between SVL and maximum jump length at intermediate temperature (where frogs jumped furthest). Statistical analysis was performed using Statistica<sup>TM</sup> 9.0; the level of significance was  $\alpha \leq 0.05$  for all tests. All data are presented as means  $\pm$  SD.

# RESULTS

# Measurements of $T_{e}$

 $T_{\rm e}$  and water loss were highest in the warm & dry microhabitat, where  $T_{\rm a}$ , wind speed and VDD were highest (Table 2). Water loss was lowest in the cool & wet microhabitat, while  $T_{\rm e}$  was lowest in the cool & dry habitat (Table 2).  $T_{\rm e}$  derived from the three model sizes was similar. On day 1,  $T_{\rm a}$  reached a maximum of 25.4 °C in the shade and 29.2 °C in the sun (Fig. 1), with larger temperature fluctuations in the sunny microhabitat, as this spot was either exposed to direct sunlight or occasionally shaded by small

**Table 2.** Ambient temperature ( $T_a$ ), relative humidity (RH), vapour density (VD<sub>air</sub>) and wind speed of the four different environmental categories (mean ± SD; *n* is sample size). Operative temperature ( $T_e$ ), vapour density at the frog model surface (VD<sub>skin</sub>) and water loss deriving from the physical models placed in these microhabitats are also given (mean ± SD; *n* is sample size). VDD is the vapour density deficit (difference between VD<sub>skin</sub> and VD<sub>air</sub>). VD was calculated from mean values of  $T_a$  and RH using Smithsonian meteorological tables (List, 1966).

	Ambient conditions					_	Model				
Environmental category	n	$T_{a}(^{\circ}\mathrm{C})$	RH (%)	VD <sub>air</sub> (gm <sup>-3</sup> )	Wind speed (ms <sup>-1</sup> )	n	$T_{\rm e}$ (°C)	VD <sub>skin</sub> (gm <sup>-3</sup> )	VDD (gm <sup>-3</sup> )	Water loss (g per 30 min)	
Warm & dry	3	20.53±2.38	34.73±8.24	6.20	0.20±0.35	6	20.45±2.42	17.76	11.56	0.39±0.03	
Warm & wet	3	19.30±0.75	56.47±6.62	9.37	$0.03 \pm 0.06$	6	19.42±0.91	16.72	7.34	$0.22 \pm 0.04$	
Cool & dry	3	16.17±0.75	44.97±6.56	6.20	0.07±0.12	6	16.07±0.77	13.69	7.49	0.13±0.06	
Cool & wet	4	15.33±0.46	56.57±3.06	7.41	$0\pm0$	8	17.62±1.61	15.03	7.62	0.11±0.03	



**Fig. 1.** Ambient temperatures ( $T_a$ ) in one shaded microhabitat (next to large bush, grass cover, moist sandy soil – shade, left) and one open microhabitat (no plant cover, soil covered with dry moss – sun, right), which were 3 m apart. Operative temperature ( $T_e$ ) was derived from physical frog models of different sizes (small, medium, large, N=1 each) placed in each environment.  $T_e$  was recorded continuously over one sunny, warm day (day 1, above) and one overcast, cool and windy day (day 2, below). Missing values are due to malfunctioning thermocouples or interruptions in temperature recordings during replacements of models. Similar  $T_e$  were obtained from the models when placed in the same microhabitat.  $T_e$  closely followed  $T_a$  on the cool day, but was generally lower than  $T_a$  on the sunny day.

clouds. RH averaged 47.8±2.1% (VD<sub>air</sub> 9.3±1.9 gcm<sup>-3</sup>) in the shade and 43.7±3.7% (VD<sub>air</sub> 9.6±1.9 gcm<sup>-3</sup>) in the sun. There was no wind in the shade, while wind speed was 0.1±0.1 ms<sup>-1</sup> in the sun. In both microhabitats,  $T_e$  was generally below  $T_a$ , and slightly lower in medium-sized models than in large and small ones. Day 2 was overcast and windy, and  $T_a$  was similarly low in both microhabitats (Fig. 1).  $T_a$  increased in the afternoon when the sun appeared shortly, and the temperature was higher in the shaded spot, protected from wind, than in the sunny, open spot. RH averaged 81.0±1.8% (VD<sub>air</sub> 11.6±0.5 gcm<sup>-3</sup>) in the sun. Wind speed was 0.1±0.1 ms<sup>-1</sup> in the shade and 2.4±0.6 ms<sup>-1</sup> in the open microhabitat.  $T_e$  was remarkably similar to  $T_a$  throughout the day in the shaded spot, while it was occasionally slightly higher than  $T_a$  in the open

microhabitat, with the highest  $T_{\rm e}$  obtained from the large models. Note that  $T_{\rm a}$  was only recorded hourly in the open microhabitat on day 2; it is therefore possible that brief temperature peaks may have been missed.

#### $T_{b}$ of wild frogs

The frogs that were captured over the course of the day (on the same two days that the models were placed in the two environments) were generally found in close proximity to permanent bodies of water on moist or dry soil that was covered by high grass or small bushes. No frogs could be found more than 15 m away from water or in open locations exposed to direct sunlight. Thus, frogs were found in similar microclimates and had similar  $T_b$ throughout the day.  $T_b$  of all 29 captured individuals averaged 18.3±1.5 °C (range 15.4–21.2 °C) at a mean  $T_a$  of



**Fig. 2.** Proportion of time six *Rana temporaria* spent in the cold ( $\leq$ 12 °C) and warm (>12 °C) end of a thermal gradient (mean + SD). Frogs were tested in two experimental series with different moisture content of the substrate: 1) wet and 2) wet at the cold end and dry at the warm end. When the entire gradient was wet, frogs spent significantly more time at the warm end, while they divided their time more equally between cold and warm end when the warm end of the thermal gradient was dry. Statistical results derive from a paired *t*-test (\*\**P*<0.01).

19.6±1.4 °C (range 18.0–22.8 °C). Wind speed was generally low, averaging 0.2±0.3 ms<sup>-1</sup> (range: 0–0.9 ms<sup>-1</sup>), and RH was 71.0±9.8% (range 47.7–82.9%). VD<sub>air</sub> averaged 11.9±1.2 gcm<sup>-3</sup>, VD<sub>skin</sub> 15.3±2.1 gcm<sup>-3</sup>, and VDD was 3.4±1.4 gcm<sup>-3</sup>. Field  $T_{\rm b}$  were independent of body size (ANCOVA:  $F_{2,23}$ =2.04, P=0.15), but depended on VD<sub>skin</sub> ( $F_{1,23}$ =8.86, P<0.01) and wind speed ( $F_{1,23}$ =4.83, P=0.04). Field  $T_{\rm b}$  was further affected by  $T_{\rm a}$ , although this was not statistically significant ( $F_{1,23}$ =3.59, P=0.07).

#### **Temperature preference**

Frogs placed in the gradient covered with wet sand spent more time in the warm compartments (>12 °C) than in cold ones (paired *t*-test:  $t_5 = -6.19$ , *P*<0.01; Fig. 2). They chose mean substrate temperatures of 19.4±1.7 °C. After choosing a particular  $T_a$ , all frogs stayed at that temperature for the remainder of the hour of observation. Frogs placed in the gradient with wet sand at the cold end and dry sand at the warm end showed more variation in selected temperatures (Levene's test: P<0.001), moving more often between the cold and the warm ends of the gradient. Thus, they divided their time approximately equally between the cold end and the warm end of the gradient (paired *t*-test:  $t_s = -1.43$ , *P*=0.21; Fig. 2). When stationary, frogs chose lower temperatures (17.6±2.5 °C) on wet/ dry sand than on wet sand only (paired *t*-test:  $t_5 = -2.63$ , P=0.04).

#### The effect of $T_{b}$ on locomotion

 $T_{\rm b}$  of the 13 frogs prior to jumping differed significantly between the three temperature categories (RM-ANOVA:  $F_{2,24}$ =336.56, P<0.001), with  $T_{\rm b}$  being slightly higher than the temperature recorded in the air or water that surrounded the frogs. The ambient conditions inside the jumping arena were not controlled;  $T_a$  was similar (20.1–21.3 °C), while RH increased (from 47.2% to 65.0%; VD<sub>air</sub> 8.6-11.3 gcm<sup>-3</sup>) from the warm to the cold category. Wind speed was very low for tests of all temperature categories (<1.00 ms<sup>-1</sup>). The mean jump length of the frogs differed significantly between the three temperature categories (RM-ANOVA: *F*<sub>2,24</sub>=19.45, *P*<0.001; Fig. 3), with jump length being lower in the cold than at intermediate and high temperatures (Tukey HSD test: P<0.01). Two of the 13 frogs repeatedly did not jump at all at the lowest  $T_{\rm b}$  (6.3 °C), but were not excluded from the analysis because the statistical results did not change when these two individuals were omitted. Jump length was higher in the medium category than in the warm category, but this was only marginally significant (P=0.05 after Bonferroni correction). The temperature coefficients for the jump lengths of *R. temporaria* were determined as:  $Q_{10}$  $(6.3-14.6 \text{ °C}) = 1.99; Q_{10} (14.6-22.8 \text{ °C}) = 0.80; Q_{10} (6.3)$ -22.8 °C) = 1.27. Similarly to mean jump length, maximum jump length also differed between the temperatures (RM-ANOVA:  $F_{2,24}$ =6.15, P<0.01), being higher in the medium and warm categories than in the cold (Tukey HSD test: P < 0.02). The maximum jump length at intermediate temperatures was not significantly related to SVL (linear regression:  $F_{1,11}$ =0.65, P=0.53, R<sup>2</sup>=0.04; Fig. 4).



**Fig. 3.** Jump length of 13 Rana temporaria at different body temperatures (mean  $\pm$  SD). Frogs jumped the farthest at the intermediate  $T_{\rm b}$  of 14.6 °C. Statistical results derive from a Tukey HSD test that followed a RM-ANOVA (\**P*≤0.05, \*\**P*≤0.01, \*\*\**P*≤0.001).



Fig. 4. The maximum jump length of 13 Rana temporaria at an intermediate temperature, in relation to snout-vent length (SVL). Each frog was tested only once at this temperature. Maximum jump length was not significantly related to body size (see text for statistical results).

## DISCUSSION

#### The use of physical models to determine T

In earlier studies, the rates of cutaneous water loss and estimates of  $T_{e}$  derived from agar models matched field  $T_{\rm b}$  of amphibians (Spotila & Berman, 1976; Navas & Araujo, 2000). It should be noted, however, that there are differences in absorptivity between the transparent agar models and the green/brownish colour of frogs. To test the accuracy of our models and the possible effect of differences in absorptivity, we compared the  $T_{\rm b}$  of agar models and live frogs placed in the same microhabitat, and found that they were very similar. These results suggest that the thermal properties of agar models and R. temporaria are comparable. Thus, agar models provide an accurate measurement of  $T_{a}$  and are useful in increasing our understanding of amphibian physiology and ecology. However, to overcome the possible effect of differences in absorptivity between agar models and frogs, future studies could either colour the agar or use plaster models, which can be coloured to match the absorptivity of amphibian skin and provide similar results to agar models (Tracy et al., 2007).

As expected, frog  $T_e$  was highest in warm and dry environments and lowest in cool and dry environments. In the cool environments,  $T_e$  was lower in the dry than in the wet microhabitat, possibly due to lower RH (and lower vapour density deficit between air and frog surface) and higher wind speed in the dry microhabitat, resulting in more heat loss. Water loss was higher in the warm than in the cool environments, indicating that cool microhabitats should be more favourable for amphibians that need to reduce their EWL. Water loss was higher in the dry environments than in the wet ones, as water is lost

faster to dry soil and dry air than to moist soil and humid air. Our models always lost mass, probably because of a very small osmotic gradient between the agar model and the substrate. Live frogs, on the other hand, can absorb water from the surrounding environment when in water, on moist soil, or during rain (Walker & Whitford, 1970; Wells, 2007).

 $T_{\rm e}$  was generally lower than or equal to  $T_{\rm a}$ . However, when the sky was overcast and the weather cooler, the  $T_{\rm e}$ obtained from some models was higher than  $T_{\rm a}$ . The substrate these models were placed on may have been slightly warmer than the air, as  $T_{\rm a}$  was higher before our experiment and the soil may have stored heat and increased in temperature.  $T_{\rm e}$  was similar in the different sizes of model, with no particular size absorbing more heat than others.

#### Thermal preferences of R. temporaria

According to  $T_{e}$  estimates and water loss of the agar models, frogs would be expected to select cool and wet habitats, protected from sun and wind by plant cover, as their water loss is minimal in this microclimate. Wild frogs that we captured over the course of the day were generally found under plant cover, in cool and often moist microhabitats, and never in the direct sun. Thus, frogs maintained similar  $T_{\rm b}$  throughout the day, and  $T_{\rm b}$  was also similar between different body sizes and light and dark individuals. Coloration also had no effect on field  $T_{\rm L}$  in an earlier study, where a high-altitude population of R. temporaria also showed active thermoregulatory behaviour:  $T_{\rm b}$  was higher than  $T_{\rm a}$  during the day due to the frogs basking in the sun, and at night, frogs retreated to a pond where the water acted as a temperature buffer during the nocturnal drop in  $T_a$  (Vences et al., 2002).

In addition to using direct field observations of temperature, an ectotherm's preference for favourable microhabitats can also be determined in the laboratory by placing the animal in thermal gradients (for a review see Brattstrom, 1979). In such a temperature gradient, our frogs preferred intermediate temperatures (19.4 °C) on wet substrates, probably due to the fact that they would dehydrate quicker at higher temperatures and face hypothermia at lower temperatures. When the frogs were faced with a trade-off between heat and humidity, with only the cold end of the gradient covered with wet substrate, they chose even lower temperatures (17.6 °C) and spent more time in the cold end of the gradient (Fig. 2), suggesting that lowering  $T_{\rm b}$  is less harmful than dehydration for these frogs. Similarly, toads (Anaxyrus (Bufo) woodhousei) select lower temperatures on dry than on wet sand to avoid desiccation (O'Connor & Tracy, 1992). Rapid heat loss at the cool end and water loss at the hot and dry end caused our frogs to move more often between the cool and warm segments. The frogs may have had to leave the cool end to raise their  $T_{\rm b}$ , but then returned to the cool and humid microclimate to avoid desiccation.

Behavioural hypothermia in ectotherms is defined in the literature as the phenomenon of seeking lower temperatures to reduce EWL or energy consumption (Tracy et al., 1993), and has been observed in toads, *Anaxyrus* (*Bufo*) americanus (Tracy et al., 1993) and lizards, *Sceloporus undulatus* (Crowley, 1987). The lower  $T_{\rm b}$  reduces the difference between water vapour density in the environment and at the skin, thus slowing the rate of EWL (Tracy et al., 1993). This may be important for *R. temporaria*, which tend to dehydrate quickly due to their relatively small body size and high evaporation rate through the skin (Lillywhite, 2006). In a habitat-like experimental environment with different  $T_a$  (8–31 °C), Sinsch (1984) found that R. temporaria exhibited behavioural cooling in the heat (by staying in water or cool hiding places and using evaporative cooling in dry microclimates), while warming behaviour was demonstrated in the cold. At the same time, these frogs changed their preferred time of activity from the night at high temperatures to the day at low temperatures, thus maintaining a fairly constant  $T_{skin}$ at all  $T_a$ . The frogs preferred moderate  $T_a$  (10–20 °C), and lengthy exposure to more than 30 °C led to 100% mortality (Sinsch, 1984). If shaded and moist areas provide the optimal habitat for the regulation of body temperature, R. temporaria may be affected by human-induced landscape alteration and fragmentation. It remains to be investigated to what extent the local distribution of these frogs is influenced by a reduction in shadow refuges, such as caused by deforestation and removal of natural vegetation leading to open canopy ponds.

## The effect of $T_{\rm h}$ on locomotion

 $T_{\rm b}$  had an effect on the jump performance of *R. temporaria*. We measured average jump lengths of the frogs, as we were interested in the average locomotor performance of the frogs at a particular temperature. While the maximum jump length indicates how well individuals are able to escape from predators, average jump length may also indicate how well the frogs are able to select microhabitats, and find food and mates. Frogs in our study had longer jumps at higher  $T_{\rm b}$  than at 6 °C, and jumped farthest at 15 °C (although only marginally significantly further than at 23 °C). Navas et al. (1999) reported very similar jump lengths for *R. temporaria* at comparable body temperatures, and reported an increasing jump length up to 20 °C.

Jump length of common frogs was halved at low  $T_{\rm b}$  $(\leq 6 \ ^{\circ}C)$  in both the present study and the Navas et al. (1999) study, compared to 15 °C. In addition, two of our frogs repeatedly did not jump at all at the lowest temperature. This suggests that 6 °C is approaching the frog's critical thermal minimum, at least for movement on land. Tattersall & Boutilier (1999) found that R. temporaria was capable of swimming at temperatures as low as 1.5 °C, but swimming speed and distance were significantly reduced compared to 7 °C. Critical thermal minima, defined as the temperature at which an animal has lost the ability to escape as temperatures fall to lethally low levels (Cowles & Bogert, 1944), have been reported to be between 2 and 7 °C for ectotherms, including frogs (Christian et al., 1988), lizards (Gvoždík & Castilla, 2001) and snakes (Doughty, 1994). However, lizards are rarely active at near-threshold  $T_{\rm b}$  (Huey & Stevenson, 1979) and frogs are also known to hibernate in burrows or under water during winter when  $T_{a}$  is low (Irwin et al., 1999; Roots, 2006). The ecological relevance of locomotor performance at 6 °C is therefore uncertain.

Frogs jumped the farthest at a  $T_b$  of 15 °C, suggesting that a  $T_b$  around 15 °C maximizes locomotor performance, although smaller temperature intervals should be tested to clearly define the performance plateau. This also indicates that the temperatures chosen by the frogs in the thermal gradient experiment are ecologically relevant, and optimized (in a broad sense) their locomotor performance. We did not measure jump lengths at higher  $T_b$ , as field  $T_b$ was not found to be higher than 22 °C in our study. Future experiments should cover a wider temperature range to determine the critical thermal minimum and maximum of these frogs.

The temperature dependence of jump performance has also been shown in other amphibians. Cricket frogs (Acris crepitans), for instance, jumped equally well at temperatures of 23 and 30 °C, but frogs produced shorter jumps at 15 °C (Walvoord, 2003). Maximum jump distance of Lithobates (Rana) pipiens was lowest at 14 °C, increased with increasing temperature, reached a maximum at 25 °C, and decreased thereafter (Hirano & Rome, 1984). The maximum jump distance of Cuban tree frogs (Osteopilus septentrionales) increased over a range of  $T_{\rm b}$ from 11 to 30 °C (Peplowski & Marsh, 1997). Knowles & Weigl (1990) tested five frog species, whose maximum jump length increased from 5 °C up to 20 or 30 °C. However, Rana clamitans and Lithobates (Rana) sylvaticus showed narrow thermal optima, whereas the other three species (Acris crepitans, Hyla femoralis and Pseudacris triseriata) showed wider performance plateaux. In the field, amphibians select a  $T_{\rm b}$  that allows optimal locomotor performance, as shown in cricket frogs (Walvoord, 2003).

#### Conclusion

 $T_{\rm b}$  affects the locomotor performance of *R. temporaria*, and frogs showed maximal jumping distances at an intermediate temperature (15 °C). Field  $T_{\rm b}$  ranged from 15 to 21 °C, even on a very warm summer day, reaching a  $T_{a}$  of 30 °C in the sun. This suggests behavioural temperature regulation. Frogs were not found in microhabitats with  $T_{a}$  higher than 23 °C, indicating that they actively avoid high temperature microhabitats in the environment. Agar models demonstrated that water loss is higher at high temperatures and in dry microhabitats, and the selection of intermediate temperatures and moist environments thus prevents extensive water loss. Amphibians generally prefer warm and moist environments, but may face a trade-off as these are not always together in the wild. Our results from the thermal gradient suggest that temperature preferences of R. temporaria are affected by the moisture content of the surrounding environment, and that frogs alternate between cool moist and warm dry microhabitats. In maintaining water balance and choosing optimal temperatures, these frogs optimize their physiological and behavioural performance.

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