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Thermal and moisture habitat preferences do not maximize jumping performance in frogs

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Summary

1. Amphibians are suffering population declines globally, and understanding how environmental parameters influence their thermal and moisture preferences and performance at various tasks is crucial to understanding how these animals will be influenced by climate change.

2. Body temperature and hydration affect organismal performance at many fitness-related tasks. Since amphibians are ectotherms with highly water-permeable skin, environmental temperature and moisture directly affect their body temperature and hydration. Therefore, amphibians should select habitats with the optimal combination of temperature and moisture to perform tasks necessary for survival. However, interactions between environmental temperature and moisture can influence habitat selection and task performance in different and often unpredictable ways, and this has only infrequently been considered.

3. We tested for interactions between environmental temperature, moisture and organismal hydration on temperature and moisture preferences and jumping performance in green frogs (*Lithobates clamitans*) in the laboratory, using thermal and moisture gradients, and high-speed video and force plate data. We then integrated the laboratory experiments with field data.

4. In the thermal and moisture gradients, frogs selected environmental conditions that minimized cutaneous evaporative water loss, hydroregulating more stringently than thermoregulating. These results are consistent with frogs in the field, which had highly variable body temperatures, but were always hydrated above 95% of their standard mass. However, conditions that minimized evaporative water loss frequently did not maximize jumping performance because warmer temperatures conferred greater performance.

5. The ecology of *L. clamitans* may explain the discrepancy between their preferences and jumping performance optima because the frogs remain in wet environments that serve as refuges from dehydration. In parts of their range where frogs are subjected to warmer and drier conditions, they are likely to select microhabitats that minimize the risk of dehydration, possibly at the expense of their ability to forage and escape from predators.

Key-words: amphibian, hydration, locomotion, temperature

Introduction

Temperature and water influence virtually every aspect of organismal biology from providing the proper conditions for biochemical reactions (Angilletta, Steury & Sears 2004; Edwards, Jenkins & Swanson 2004; Stocker, Keith & Toney 2004; Roufayel, Biggar & Storey 2011) to determining the range and abundance of species (Sexton, Phillips & Bramble 1990; Lillywhite & Navas 2006; Buckley & Jetz 2007; Monzón, Moyer-Horner & Palamar 2011). Nevertheless, our understanding of temperature and moisture preferences and how these factors influence an organism's ability to perform fitness-related tasks is hampered because of complex interactions between environmental temperature and moisture (Preest & Pough 1989; Kearney *et al.* 2012).

Despite these interactions, most research on how temperature and moisture affect organismal function considers them separately. From this work, we know that temperature influences organismal function and behaviour by changing rates of underlying biochemical reactions (Angilletta, Steury & Sears 2004; Glanville & Seebacher 2006). Maintaining optimal body temperature for task performance is important for fitness (Lovegrove 2003; Kingsolver & Huey 2008; Podrabsky, Clelen & Crawshaw 2008; Kingsolver *et al.* 2011) and a challenge for ectotherms because they rely on environmental temperature to

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regulate their body temperature (Angilletta 2009). Ectotherms generally prefer warmer temperatures, at which task performance tends to be optimal (Kingsolver & Huey 2008). However, thermal preferences and optima differ with the task being performed (Huey & Stevenson 1979; Martin & Huey 2008), intra-individual thermal sensitivity (Buttemer 1990; Wells 2007; Williams *et al.* 2008) and environmental variables, such as moisture availability (Köhler *et al.* 2011).

As with temperature, environmental moisture and changes in hydration also affect task performance (Crowley 1987; Moore & Gatten 1989; Ladyman & Bradshaw 2003; Plummer *et al.* 2003). Generally, performance decreases as an organism becomes dehydrated (Preest & Pough 1989; Weinstein 1998; Rogowitz, Cortes-Rivera & Nieves-Puigdoller 1999), and performance tasks differ in their sensitivity to changes in hydration (Moore & Gatten 1989; Tingley, Greenlees & Shine 2012). Since biochemical reactions take place in aqueous solution, maintaining optimal hydration is critical to providing optimal solute concentrations for these reactions (Wolcott & Wolcott 2001; Lillywhite & Navas 2006). Organisms that are particularly water permeable do this by frequent rehydration (Feder & Londos 1984; Tracy, Laurence & Christian 2011) or reduction in bodily water loss (Wolcott & Wolcott 2001; Tracy, Christian & Tracy 2010).

Body temperature and hydration affect organismal behaviour and task performance for animals as varied as mammals, snakes, turtles, amphibians, crabs and insects (Weinstein 1998; Ladyman & Bradshaw 2003; Lovegrove 2003; Plummer *et al.* 2003; Stocker, Keith & Toney 2004; Kingsolver *et al.* 2011). Amphibians are particularly ideal for studying interactions between temperature and moisture on organismal biology because they are both ectotherms and have more permeable skin than other tetrapods (Duellman & Trueb 1986; Lillywhite 2010). Therefore, environmental temperature and moisture directly affect amphibian body temperature and hydration, which affect their environmental preferences and task performance (Shoemaker, Baker & Loveridge 1989; Preest & Pough 2003; Walvoord 2003; Köhler *et al.* 2011). Some amphibians reduce cutaneous evaporative water loss (CEWL) through adaptations, such as mucus production and assuming a water-conserving posture (Lillywhite *et al.* 1997; Wolcott & Wolcott 2001). They can also increase CEWL to reduce body temperature when it is high (Wygoda 1988; Prates & Navas 2009). Given the interactions between temperature and moisture, and that both of these factors affect organismal performance, one would expect thermal and hydration effects on performance to interact, and there is some evidence for this in amphibians (Preest & Pough 1989; Weinstein 1998; Rogowitz, Cortes-Rivera & Nieves-Puigdoller 1999; Niewiarowski *et al.* 2008; Titon *et al.* 2010).

The interactions between temperature and moisture dictate that both must be considered when studying animal task performance and environmental preferences (Walvoord 2003; Navas, Gomes & Carvalho 2008; Williams *et al.* 2008). Interactive effects of temperature and moisture are also important for studies of animal conservation in the light of current changes in climate (McMenamin, Hadly & Wright 2008). Due to their highly permeable skin, amphibian development, survival, reproduction and persistence may be disproportionately affected by changes in the thermal and moisture environment (Rohr & Madison 2003; McMenamin, Hadly & Wright 2008). Interspecific differences in skin permeability and adaptations to different habitats may influence amphibian responses to environmental temperature and moisture changes (Schmid 1965; Prates & Navas 2009). For instance, thermal sensitivity of performance may be reduced by dehydration in some species (Preest & Pough 1989), while sensitivity of performance to dehydration may be more severe at certain temperatures for certain species (Titon *et al.* 2010).

Here, we assess the influence of temperature and moisture interactions on frog jumping performance, which is their primary mode of locomotion, foraging and predator escape, and test whether thermal and moisture preferences in the laboratory and the field coincide with thermal and hydration optima for jumping performance. We used *Lithobates clamitans* (Latreille, 1801) because they are primarily aquatic in the field and less tolerant of dehydration than more terrestrial species (Schmid 1965; Conant & Collins 1998), so we expect them to be particularly sensitive to changes in moisture availability in laboratory experiments. We tested the hypotheses that (i) *L. clamitans* would favour environmental temperature and moisture combinations that reduce CEWL; (ii) *L. clamitans* in nature would hydroregulate more strictly than they thermoregulate because they are adapted to an aquatic habitat; and (iii) *L. clamitans* would have maximal jumping performance at high body temperatures when hydrated, but the thermal performance optimum and thermal sensitivity for jumping would decrease for dehydrated frogs.

Materials and methods

EXPERIMENTAL ANIMALS

We collected 16 *L. clamitans* from the Westborough Wildlife Management Area, Massachusetts, USA. We housed animals individually in 37 × 22 × 22 cm (L × W × H) terraria with leaf litter substrate and large water dishes. We kept the frogs at 22 ± 3 °C in a natural light–dark cycle and fed them crickets three times a week.

We conducted all laboratory trials from February 2012 to November 2013, between 6:00 and 18:00 h. We fasted the frogs for 3 days prior to each trial to avoid changing their thermal preferences due to digestion (Sievert & Andreadis 2002; Preest & Pough 2003). We did not dehydrate frogs more than once a week. We monitored frog mass weekly and eliminated frogs we deemed unhealthy due to mass loss or external symptoms from all experiments. We were also only able to use frogs large enough for good quality data collection from the force plate for performance experiments. This resulted in samples of 13 frogs (range: 5·5–68·3 g; mean ± SD: 34·4 ± 12·9 g) for preference experiments and nine frogs (range: 24·5–52·7 g; mean ± SD: 40·7 ± 8·36 g) for performance experiments. All care and procedures were approved by the Clark University Institutional Animal Care and Use Committee.

TEMPERATURE AND MOISTURE PREFERENCE EXPERIMENTS

Experimental design

To quantify temperature and moisture preferences of the frogs, we ran two experiments in parallel. In the first, we placed either fully hydrated or dehydrated frogs in a thermal gradient that had either a wet or dry substrate. We expected frogs to select the highest body temperatures on a wet substrate regardless of hydration state, and the lowest body temperatures when dehydrated and on a dry substrate. In the second, we placed fully hydrated or dehydrated frogs in a moisture gradient that was at 10, 20 or 30 °C. We expected frogs to select the highest moisture level at 30 °C when dehydrated to reduce CEWL. We conducted three trials of each experiment with each frog when fully hydrated and twice when dehydrated to reduce stress on the frogs from repeated dehydration.

We also ran control trials on a subset of hydrated individuals with uniform temperature in the temperature gradient apparatus and uniform moisture in the moisture gradient apparatus to ensure that individuals were not selecting positions in the gradient due to uncontrolled factors. In both gradients, these individuals remained in the middle half of the lengths of the gradients throughout these trials, avoiding the ends. This contrasts with experimental trials, where individuals often frequented the extremes of the gradients, indicating a lack of bias in the experimental results.

Prior to trials at full hydration, we placed the frogs in 2 cm of distilled water for 30 min to ensure they were fully hydrated (Moore & Gatten 1989). We patted the frogs dry with paper towel and emptied their bladders using a plastic catheter (Hollister Apogee Female Intermittent Catheter, Libertyville, IL, USA; Moore & Gatten 1989; Preest & Pough 2003). We then measured their standard mass (Ruibal 1962) with an electronic scale (Ohaus Scout Pro SP401, Pine Brook, NJ, USA) to the nearest 0·1 g. Prior to trials in which frogs were dehydrated, we calculated standard mass as described above, but then allowed the frogs to dehydrate to 80–85% of standard mass at room temperature in mesh cages (Moore & Gatten 1989), monitoring their hydration level hourly.

We allowed frogs to acclimate to each gradient for 30 min before we began trials. We performed thermal preference trials in a glass terrarium (68 × 14 × 22 cm) insulated with Styrofoam. We used blocks of ice and a 100-W ceramic infrared heater (Zoo Med Repticare CE-100, San Luis Obispo, CA, USA) at opposing ends of the terrarium to obtain a gradient of 5–35°C. We used paper towels as a substrate to provide traction for the frog, and these were either dry or wet with 0·5 cm of distilled water. We ran trials for 2 h, taking observations every half-hour (four observations per trial per frog). We measured body temperature to the nearest 0·5 °C from the dorsal surface of each frog using an infrared thermometer (Raytek MiniTemp FS, Santa Cruz, CA, USA).

We conducted moisture preference trials in a plastic moisture gradient (78 × 20 × 21 cm) with six compartments (each 13 × 20 × 17 cm). We filled each compartment with oven-dried peat moss, keeping the first compartment dry and wetting each adjacent compartment with 15%, 30%, 45%, 60% and 75% distilled water by volume. We placed the moisture gradient in a 122 × 32 × 48 cm, Styrofoam-insulated aquarium filled with 15 cm of water. We kept the moisture gradient at 20 °C; heated it to 30 °C using two 250-W aquarium heaters (Pacific Coast Imports QH-250, Woodburn, OR, USA) and 12-W radiant heaters (The Bean Farm Ultratherm Heat Pads, Carnation, WA, USA); or cooled it to 10 °C by placing the gradient in a cold room at 2 °C overnight and then adding ice to the aquarium. We noted the compartment in which we observed the frog every 15 min for 2 h, totalling eight observations per trial.

Since frogs can assume a water-conserving posture that minimizes the surface area exposed to the air when under threat of dehydration (Wolcott & Wolcott 2001; Wells 2007; Navas, Gomes & Carvalho 2008), we noted the frog's posture at every observation while in the gradients. We coded posture (Fig. 1) as (i) water-conserving posture where the frog tucked its limbs against its body and flattened its head against the substrate; (ii) a posture where the frog pulled its limbs towards the body but lifted its head; (iii) an alert position where most of the ventral surface was elevated and the limbs were not tucked; and (iv) an active posture where the frog was moving.

Statistical analyses

Since we had data for multiple trials per individual in each treatment, we calculated the mean and mean variance of all body temperature and moisture-level observations for each frog in each treatment combination to eliminate pseudoreplication. We performed all statistical analyses in R v2.15 (R Development Core Team 2012). We conducted repeated-measures ANCOVAS on the mean and variance of the selected body temperature and moisture level, with frog hydration state, substrate moisture or temperature (for thermal and moisture gradients, respectively) as fixed factors, and included their interaction. We also included standard mass as a covariate.

We performed a randomization repeated-measures ANCOVA for each analysis with 10 000 randomizations of the data (see Appendix S1, Supporting information) because some of the variables were not normally distributed or homoscedastic (Petraitis, Beaupre & Dunham 2001). These randomization ANCOVAS gave the same qualitative results as standard ANCOVAS, but provided higher power. We ran *post hoc* pairwise paired-sample *t*-tests for each significant factor and corrected for multiple comparisons using the Benjamini–Hochberg (BH) method (Benjamini & Hochberg 1995; Williams, Jones & Tukey 1999).

We analysed the posture frequency data using *G*-tests (Sokal & Rohlf 1995). We first tallied the observations of each posture in each treatment level and combination of treatment levels for each gradient, using only the first two trials for the treatments at full hydration to maintain equal numbers of observations between treatments. We divided the total number of posture observations by the number of frogs ($n = 11$) to reflect our actual sample size. We then performed *G*-tests comparing the frequency of selected postures between hydrated and dehydrated frogs in each gradient, and that between frogs on wet and dry substrates in the thermal gradient, and among frogs at 10, 20 and 30 °C in the moisture

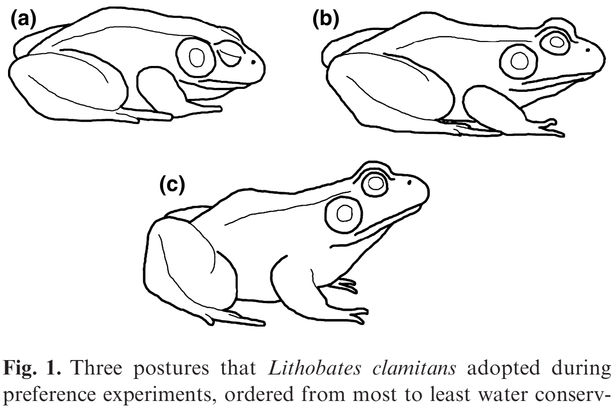

Fig. 1. Three postures that *Lithobates clamitans* adopted during preference experiments, ordered from most to least water conserving. Posture 4 is a moving frog (not shown).

gradient. We also tested for heterogeneity among treatment-level combinations to account for interactions between factors. We selected *a priori* comparisons of treatment-level combinations, so did not correct for multiple comparisons. Specifically, in the thermal gradient, we compared postures assumed by hydrated frogs on a wet substrate to each of the other treatment combinations. In the moisture gradient, we compared postures assumed by hydrated frogs at 20 °C to each of the other treatment combinations. We corrected these analyses for small sample size using Williams' correction (Sokal & Rohlf 1995) and did all *G*-tests by hand.

FIELD BODY TEMPERATURE AND HYDRATION

Experimental design

We collected field data from June to September 2013 between 6:00 and 18:00 h in the Westborough Wildlife Management Area. This sampling period included warm and wet conditions in the summer and cooler and drier conditions in the autumn, allowing us to quantify temperature and moisture preferences over varied climatic conditions. We visited eight different vernal pools and streams, sampling from a different location each day to avoid resampling individuals. We collected environmental temperature and relative humidity data at each site using a handheld weather station (Kestrel 4000 Pocket Weather Tracker; Nielsen-Kellerman, Boothwyn, PA, USA).

We caught 1–12 individuals per site, for a total of 75. We only collected frogs that were not actively foraging or calling and were not in a body of water. We recorded the body temperature of each frog with the infrared thermometer, captured the frog, emptied its bladder using a catheter and measured its mass with a spring balance (Pesola, Baar, Switzerland) to the nearest 1 g. We allowed the frog to fully hydrate by placing it in a container with 2 cm of distilled water for 15 min (Preest, Brust & Wygoda 1992), which is sufficient time for a frog to fully rehydrate (Walvoord 2003). We then weighed the frog again to obtain standard mass and calculated hydration by dividing mass at capture by standard mass (Preest, Brust & Wygoda 1992). We housed frogs individually until the completion of the field observations for each site to avoid resampling individuals.

Statistical analyses

We used frog body temperature, frog hydration, air temperature and relative humidity at the site to investigate frog thermal and moisture preferences in nature. We performed nonparametric Spearman's correlations between all frog and environmental variables since some of the variables were not normally distributed (Sokal & Rohlf 1995). To determine whether frogs thermoregulate in nature, we performed a Wilcoxon signed rank test (Sokal & Rohlf 1995) between frog body temperature and environmental temperature at capture site to determine whether these variables significantly differed. Thermoconforming frogs would have body temperatures not different from environmental temperatures.

JUMPING PERFORMANCE EXPERIMENTS

Experimental design

We induced frogs to jump from a force plate at different combinations of body temperature and hydration to test for the effects of these factors on jump performance. We expected the thermal optimum for jump performance to be highest in hydrated frogs, while we expected dehydrated frogs to have lower thermal optima and lower thermal sensitivity in jumping performance (Preest & Pough 1989; Walvoord 2003). We jumped each frog at each combination of hydration (100%, 87% and 75%) and body temperature (10, 20 and 30 °C) at least twice (median = 4, range 2–4). We then selected the jump with the highest maximum acceleration for each frog and treatment for analysis. We dehydrated frogs to 87% and 75% of standard mass, as described above. Once the frog reached the desired hydration, we placed it in a damp plastic container and in an incubator (Tritech Research, Inc., Los Angeles, CA, USA) for at least 30 min to allow the frog to reach the desired body temperature while maintaining the desired hydration.

We placed each frog on a 12 × 12 × 11-cm hollow aluminium platform mounted onto a multicomponent piezoelectric force plate (Type 9260AA3; Kistler Group, Winterthur, Switzerland) with fibreglass screening glued to the top of the platform for traction. The platform enticed the frogs to jump rather than hop. We induced frogs to jump by lightly prodding their posteriors with a brush. We measured body temperature of each frog immediately following each jumping trial with an infrared thermometer. We also recorded each jump from lateral view using a high-speed video camera (TroubleShooter LE; Fastec Imaging Corporation, San Diego, CA, USA). The force plate and video camera were synchronized and set to sampling rates of 2500 and 250 Hz, respectively. We only included jumps in which the frog fully extended its hind legs, jumped horizontally in plane with the camera, lacked false starts and produced clear force data. We then used the force and video data to calculate maximal acceleration, velocity at take-off, angle of take-off, time to peak acceleration and jump distance (following Toro *et al.* 2003; see Appendix S1 for a detailed description).

Statistical analyses

We did a randomization repeated-measures two-factor fully crossed ANCOVA with 10 000 replicates on each of the five jump parameters, as described above. In each of these analyses, we used treatment temperature (10, 20 and 30 °C) and frog hydration (75%, 87% and 100% of standard mass) as fixed factors and standard mass as a covariate. Since body temperature changed when the frogs were removed from the incubator, we repeated the analysis with body temperature as a covariate, but do not include these results because they were qualitatively identical. We performed *post hoc* paired-sample *t*-tests on all pairwise combinations of treatment levels for main effects that were significant. For significant interaction terms, we considered only a subset of possible pairwise comparisons: all comparisons among 10 °C and 75% hydrated, 10 °C and fully hydrated, 30 °C and 75% hydrated and 30 °C and fully hydrated. We corrected for multiple comparisons using the BH method (Benjamini & Hochberg 1995).

Results

TEMPERATURE AND MOISTURE PREFERENCES IN THE LABORATORY

The results from our preference experiments supported our hypothesis that *L. clamitans* select environmental temperatures and moistures that reduce CEWL. In the thermal gradient, frog hydration and whether the substrate was wet or dry affected body temperature preferences (Table 1). *L. clamitans* on wet substrates selected warmer temperatures than frogs on dry substrates, and on the dry substrate, hydrated frogs selected warmer body temperatures than dehydrated frogs (Fig. 2a). We used variance in body temperature as a measure of the precision of

Table 1. Randomization repeated-measures ANCOVA results for the thermal gradient experiment

	Mean			Variance		
Effect	MS	*F*	*P*	MS	*F*	*P*
Hydration	23·67	5·36	**0·039**	42·39	2·05	0·189
Moisture	229·40	69·22	**< 0·001**	238·09	6·27	**0·027**
Hydr × Moist	21·25	4·32	0·059	1·61	0·09	0·775
Mass	8·12	2·39	0·149	215·60	6·72	**0·029**
Residuals	3·40			27·16		

The mean and variance of selected body temperature are response variables. Frog hydration (hydrated or dehydrated, d.f. = 1) and substrate moisture (wet or dry, d.f. = 1) are fixed factors, and standard mass (d.f. = 1) is the covariate. Mean squares (MS), *F*-statistics and *P*-values are provided. Residual MS are given for each ANCOVA (d.f. = 11). Significant *P*-values are in bold.

Table 2. Randomization repeated-measures ANCOVA results for the moisture gradient experiment

	Mean			Variance		
Effect	MS	*F*	*P*	MS	*F*	*P*
Hydration	6·74	5·88	**0·032**	9·22	22·31	**< 0·001**
Temperature	0·09	0·19	0·831	2·85	12·86	**< 0·001**
Hydr × Temp	0·02	0·07	0·926	0·69	1·84	0·185
Mass	8·54	8·50	**0·014**	1·67	1·17	0·307
Residuals	1·01			1·43		

The mean and variance of selected moisture level are response variables. Frog hydration (hydrated or dehydrated, d.f. = 1) and gradient temperature (10, 20 or 30 °C, d.f. = 2) are fixed factors, and standard mass (d.f. = 1) is the covariate. Mean squares (MS), *F*-statistics and *P*-values are provided. Residual MS are given for each ANCOVA (d.f. = 11). Significant *P*-values are in bold.

thermoregulation, and this was influenced by substrate moisture and body mass (Table 1). Smaller frogs thermoregulated less precisely than large frogs ($R = -0·348$), and frogs on dry substrates thermoregulated less precisely than those on wet substrates (Fig. 2b).

Hydration and body mass, but not temperature, significantly affected moisture preferences (Table 2), with dehydrated (Fig. 3a) and smaller ($R = -0·322$) frogs preferring wetter substrates. Both frog hydration and gradient temperature significantly affected the precision of hydroregulation (Table 2). Dehydrated frogs and those in the 10 °C treatment selected moisture conditions more precisely than hydrated frogs or those at warmer temperatures (Fig. 3b).

In the thermal gradient, postures used by frogs differed significantly between substrate moisture levels ($G = 8·36$, $P = 0·039$), but not frog hydration states ($G = 4·88$, $P = 0·181$). Heterogeneity between substrate moisture and frog hydration approached significance ($G = 16·32$, $P = 0·061$), with frogs on the dry substrate using the water-conserving posture more than frogs on the wet substrate, especially when the frogs were dehydrated (Fig. 4a).

In the moisture gradient, the effect of frog hydration on posture approached significance ($G = 7·10$, $P = 0·069$), with hydrated frogs selecting posture 3 more frequently than dehydrated frogs (Fig. 4b). Gradient temperature ($G = 2·33$, $P = 0·887$) and heterogeneity between hydration and temperature ($G = 10·81$, $P = 0·766$) did not affect posture. Interestingly, frogs rarely selected the water-conserving posture or the most active posture in any treatment of the moisture gradient, but frogs in the thermal gradient adopted these postures frequently (Fig. 4).

FIELD BODY TEMPERATURE AND HYDRATION

Our results from the field supported our hypothesis that *L. clamitans* stringently hydroregulate to maintain optimal hydration and thermoregulate to reduce CEWL. *L. clamitans* body temperatures ranged from 15·0 to 32·5 °C (mean ± SD: 22·1 ± 4·1 °C), and were significantly lower than environmental temperatures ($V = 2750·5$, $P < 0·001$). Body temperature was significantly correlated with environmental temperature ($\rho = 0·68$, $P < 0·001$), but not relative humidity ($\rho = 0·25$, $P = 0·033$) after correcting for

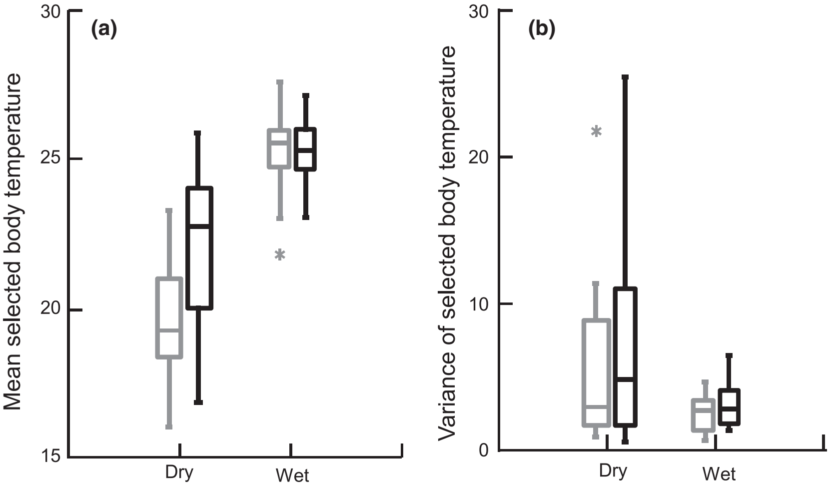

Fig. 2. Box plots for (a) mean and (b) variance of selected body temperature on wet and dry substrates in the thermal gradient. Grey boxes are for frogs dehydrated to 80% of standard mass, and black boxes are for fully hydrated frogs. Boxes show the interquartile range. Outliers ≥1·5 times the interquartile range are denoted with an asterisk.

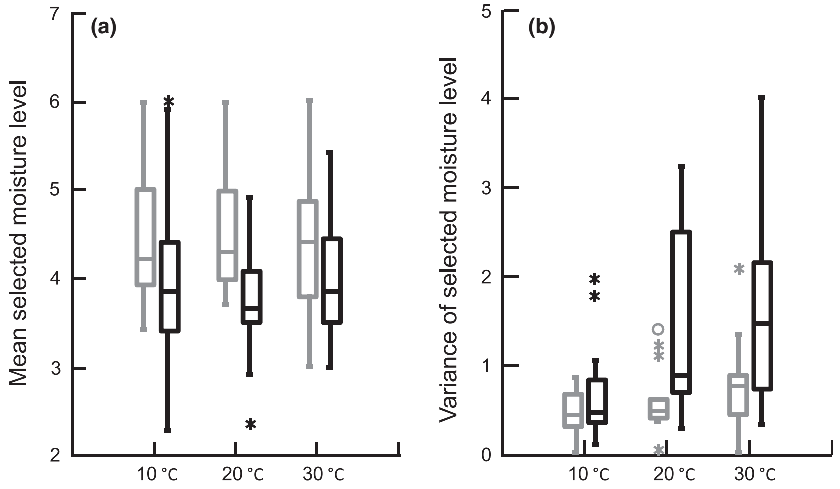

Fig. 3. Box plots for (a) mean and (b) variance of selected moisture level at three experimental temperatures of the moisture gradient. Grey boxes are for frogs dehydrated to 80% of standard mass, and black boxes are for fully hydrated frogs. Boxes show the interquartile range. Outliers ≥1·5 and ≥3 times the interquartile range are denoted with asterisks and open circles, respectively.

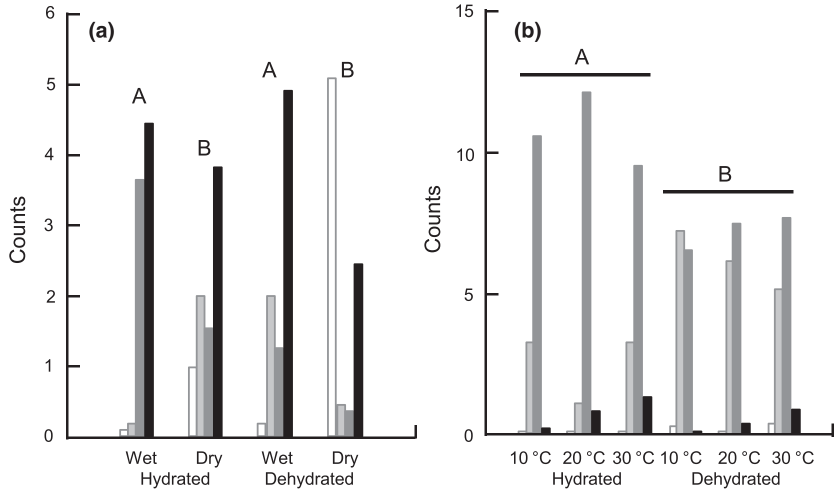

Fig. 4. Histograms of frequencies of postures adopted by frogs in the (a) thermal and (b) moisture gradients for different treatment-level combinations of frog hydration and (a) substrate moisture or (b) gradient temperature. Bars show observed counts for postures 1 (white), 2 (light grey), 3 (dark grey) and 4 (black). Capital letters denote treatments that are not significantly different from each other.

multiple comparisons. *L. clamitans* always maintained hydration above 95% of standard mass and often near 100% (mean ± SD: 99·0 ± 1·9%). *L. clamitans* hydration was not related to body temperature ($\rho = -0{\cdot}222$, $P = 0{\cdot}055$), environmental temperature ($\rho = -0{\cdot}178$, $P = 0{\cdot}126$) or relative humidity ($\rho = -0{\cdot}111$, $P = 0{\cdot}342$).

JUMPING PERFORMANCE EXPERIMENTS

Different aspects of jump performance were affected differently by frog body temperature and hydration. The interaction between body temperature and hydration significantly affected time to peak acceleration (Table 3), supporting our hypothesis of reduced thermal sensitivity in dehydrated frogs. Specifically, both hydrated and dehydrated frogs at 30 °C reached peak acceleration faster than fully hydrated frogs at 10 °C, but dehydrated frogs performed equally at 10 and 30 °C (Fig. 5c). However, we found no evidence of decreased thermal optima in dehydrated frogs.

We also found that frogs at 10 °C had significantly lower jump take-off velocities than frogs at 20 °C (Table 3, Fig. 5a). Warmer frogs also appeared to have higher jump angles (Table 3), but *post hoc* tests were not significant because they did not factor out the effects of hydration. Hydration and body temperature both significantly influenced acceleration during take-off, but did not interact (Table 3). Acceleration was lower in dehydrated frogs and those with lower body temperatures (Fig. 5b). Neither frog hydration nor body temperature affected jump distance (Table 3).

Discussion

ENVIRONMENTAL PREFERENCES DO NOT MATCH JUMPING OPTIMA

Our findings clearly show that body temperature influences almost every aspect of jumping performance more than hydration state in *L. clamitans* (Table 3). However, *L. clamitans* seems to select thermal and moisture conditions that would minimize CEWL, even when this is at odds with optimal jumping performance, in both the laboratory (Figs 2 and 3) and the field. This suggests that the costs of dehydration, including reduced blood osmolality (Ruibal 1962; Moore & Gatten 1989; Walvoord 2003) and

Table 3. Randomization repeated-measures ANOVA results for five jump parameters

	Hydration			Temperature			Hydr × Temp			
Response	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	Residual MS
Velocity	0·26	2·60	0·104	0·75	4·05	**0·043**	0·09	1·06	0·392	1·09
Angle	968·70	1·65	0·216	633·00	4·75	**0·023**	202·30	0·62	0·654	499·90
Accel	514·00	8·08	**0·004**	789·58	14·91	**0·001**	25·17	0·43	0·782	1556·00
Time to accel	<0·01	0·43	0·654	<0·01	4·33	**0·027**	<0·01	3·43	**0·020**	<0·01
Distance	0·01	0·36	0·700	0·10	2·38	0·122	0·02	0·47	0·476	0·14

For each analysis, the jump parameter is the response variable, and frog hydration (75%, 87% or 100%, d.f. = 2) and body temperature (10, 20 or 30 °C, d.f. = 2) are fixed factors. Mean squares (MS), *F*-statistics and *P*-values are provided. Residual MS are given for each ANOVA (d.f. = 8). Significant *P*-values are in bold.

activity level (Lorenzon *et al.* 1999), may outweigh the costs of decreased jumping performance, including poor predator avoidance and foraging performance (Moore & Gatten 1989; Köhler *et al.* 2011). Therefore, unlike ectotherms that are resistant to dehydration, more aquatic amphibians do not always prefer temperatures that maximize performance (Stevenson, Peterson & Tsuji 1985). Instead, they appear to behaviourally minimize rates of CEWL to maintain optimal hydration instead of optimal body temperature (Feder & Londos 1984; Duellman & Trueb 1986; Wells 2007; Oromı′, Sanuy & Sinsch 2010; Köhler *et al.* 2011).

Contrary to expectations, we also observed that cold frogs hydroregulated more precisely than warm frogs (Fig. 3). Frogs should hydroregulate more strictly at higher temperatures because of increased CEWL (Duellman & Trueb 1986; Angilletta 2009). *L. clamitans* likely reduced their activity levels in the moisture gradient because they were cold (Stevenson, Peterson & Tsuji 1985; Wells 2007; Kingsolver & Huey 2008), not because they were actively hydroregulating. Many temperate ectotherms naturally become inactive during cold weather (Wells 2007). For amphibians that inhabit more arid environments, the cost of hydroregulating at the expense of thermoregulating is even greater, as is evident in arid-dwelling arboreal frogs such as *Litoria, Chiromantis* and *Phyllomedusa*, which have evolved specialized adaptations for high physiological resistance to CEWL (Shoemaker, Baker & Loveridge 1989; Buttemer 1990).

ENVIRONMENTAL PREFERENCES ARE A PRODUCT OF NICHE USE

The highly aquatic nature and physiology of *L. clamitans* (Schmid 1965; Conant & Collins 1998) explain their temperature and moisture preferences. This species maintains high hydration levels, likely because of their constant proximity to water (Tracy, Christian & Tracy 2010). Their low tolerance to desiccation and low physiological resistance to CEWL (Schmid 1965; Duellman & Trueb 1986) imply that hydroregulation is key in maintaining these high hydration levels. In the field, *L. clamitans* had high hydration levels and body temperatures below environmental temperatures, consistent with reducing CEWL. In the laboratory, they also selected cool and wet conditions when dehydrated. These results support our first two hypotheses that these frogs hydroregulate stringently in both the laboratory and the field to limit CEWL and maintain high hydration states.

Amphibians, in general, use behavioural habitat selection and a range of physiological adaptations to reduce thermal and moisture stress (Rothermel & Luhring 2005; Young *et al.* 2005; Lillywhite 2010; Tingley, Greenlees & Shine 2012). However, differential sensitivity among amphibian species to changes in hydration and body temperature (Gatten 1987; Edwards, Jenkins & Swanson 2004; Hillman *et al.* 2011; Roufayel, Biggar & Storey 2011) suggests that thermal and moisture preferences should differ among species, which may employ different strategies to what we document here for *L. clamitans*. For example, the canyon tree frog (*Hyla arenicolor*) has high resistance to CEWL and a large bladder from which to reabsorb water (Snyder & Hammerson 1993). In the field, these frogs seek water infrequently, preferring to bask and forage (Snyder & Hammerson 1993). In contrast to *L. clamitans*, physiological adaptations to arid climates allow *H. arenicolor* to stay hydrated so that they are free to thermoregulate for optimal foraging performance (Snyder & Hammerson 1993).

An unexpected result from our work was that dehydrated *L. clamitans* rarely assumed the water-conserving posture in the moisture gradient (Fig. 4b; Spolita 1972). It is likely that the frogs immediately selected the wet side of the gradient and rapidly rehydrated, negating the need to assume a water-conserving posture (Duellman & Trueb 1986). Dehydrated frogs in the wet thermal gradient also showed more active postures than those in the dry gradient (Fig. 4a) and experienced an average gain of 16·4% body mass due to rehydration during this treatment. Rehydration rates are higher than dehydration rates due to various physiological adaptations, including a greater density of blood vessels in the ventral pelvic region, which is typically in contact with water (Duellman & Trueb 1986; Wells 2007), and changes in the expression of hormones such as prolactin, angiotensin and vasotocin, which have been shown to regulate water and electrolyte balance (Li *et al.* 2006; Saitoh *et al.* 2014;

Uchiyama *et al.* 2014). Therefore, when provided with a wet substrate, dehydrated *L. clamitans* experienced a return of more active behaviour as they rehydrated.

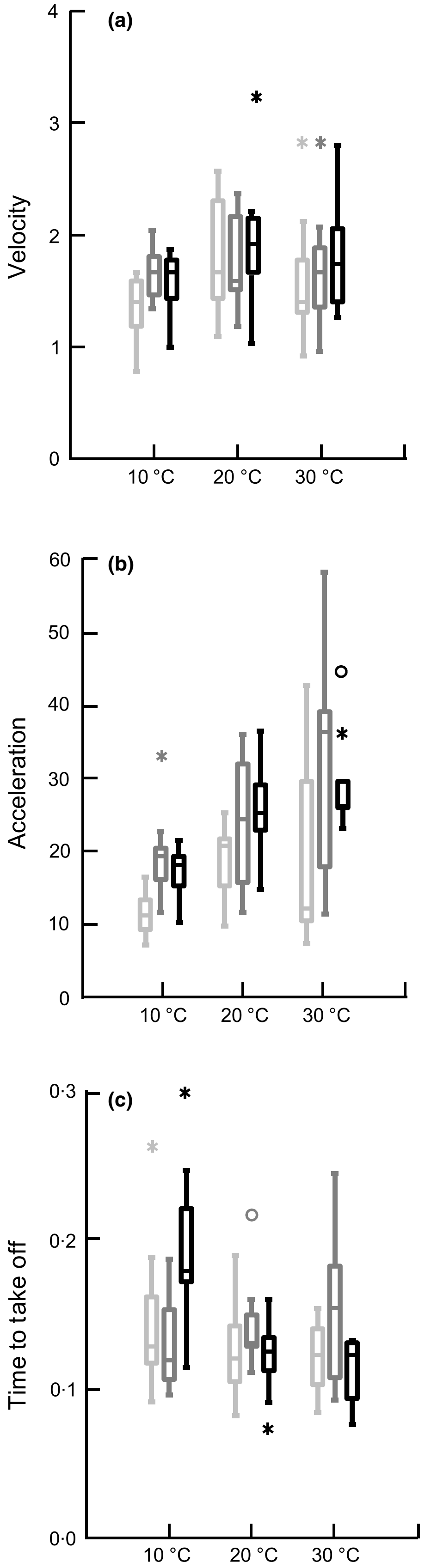

Fig. 5. Box plots for (a) velocity, (b) acceleration and (c) time to peak acceleration for different body temperatures and hydration states. Light grey, dark grey and black boxes are for frogs at 75%, 87% and 100% of standard mass. Boxes show the interquartile range. Outliers ≥1·5 and ≥3 times the interquartile range are denoted with asterisks and open circles, respectively. For velocity (a), only temperature was significant; for acceleration (b), both temperature and hydration were significant; and for time to peak acceleration (c), temperature and hydration interacted.

DO TEMPERATURE AND MOISTURE INTERACT TO AFFECT PREFERENCES AND PERFORMANCE?

Interactions between body temperature and hydration on amphibian environmental preferences and task performance dictate that considering water balance is key to understanding thermal biology and niche use (Walvoord 2003; Tracy & Christian 2005; Navas, Gomes & Carvalho 2008; Köhler *et al.* 2011). A guiding principle in ectotherm thermal biology is that hotter temperatures are better and should be preferred (Kingsolver & Huey 2008). However, dehydrated animals can have lower thermal sensitivity of various performance tasks than hydrated individuals (Gatten & Clark 1989; Moore & Gatten 1989; Weinstein 1998; Tingley, Greenlees & Shine 2012; this study). There is also a shift to lower thermal optima and upper critical limits in dehydrated animals (Preest & Pough 1989; Plummer *et al.* 2003; Köhler *et al.* 2011). Therefore, the hotter is better rule may only apply to well-hydrated animals, but this needs to be tested further on a range of taxa differing in niche use and physiological adaptations to temperature and hydration regulation.

A number of our findings clearly showed interactions between temperature and moisture affecting environmental preferences and jumping performance in *L. clamitans*. For example, substrate moisture and frog hydration affected thermoregulation (Table 1). Substrate moisture also affected posture in the thermal gradient, and temperature affected the stringency of hydroregulation (Table 2). Finally, we found reduced thermal sensitivity of time to peak acceleration in dehydrated frogs (Fig. 5c). However, many of our other jump parameters were not interactively affected by temperature and hydration (Table 3). The reasons for this may vary among parameters. For example, jump distance may be primarily determined by leg length and elastic energy storage of tendons, which do not change with temperature and hydration (Roberts & Marsh 2003; Navas, Gomes & Carvalho 2008).

Other factors, such as the metabolic pathway recruited for a task, may also influence whether it is temperature or hydration dependent (Walvoord 2003). For example, locomotor endurance relies on aerobic respiration, while burst locomotion such as jumping relies on immediately available ATP and phosphocreatine stores in muscles (Moore & Gatten 1989; Biewener 2003), which is less sensitive to temperature. Metabolic activity may shift from aerobic to anaerobic as an animal becomes dehydrated because of decreased oxygen transport by the blood (Biewener 2003;

Walvoord 2003). Since *Lithobates* species rely on anaerobic burst locomotion (Moore & Gatten 1989), dehydration may not influence jump performance as it would for animals relying more on aerobic locomotion, such as the Bufonidae (Moore & Gatten 1989; Hillman *et al.* 2014).

Many environmental factors affect an organism's biology, and these effects are modified by the organism's adaptations to the environment. In amphibians in particular, considering both temperature and moisture provides a more comprehensive understanding of the ecology of different species (Spolita 1972; Owen 1989) and their evolutionary trajectories (Lillywhite *et al.* 1997; Tingley, Greenlees & Shine 2012). Such an understanding is fundamental to conservation of this declining group of organisms (Cohen 2001; Collins & Storfer 2003; Buckley & Jetz 2007; McMenamin, Hadly & Wright 2008; McMenamin & Hadly 2010) and how they will be influenced by climate change (Owen 1989; Monzón, Moyer-Horner & Palamar 2011). Understanding the complexity of organisms' interactions with their environments is the first step to mitigating these species declines.

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Data accessibility

Data deposited in the Dryad Digital Repository http://doi:10.5061/dryad.8654h (Mitchell & Bergmann 2015).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Description and R code for randomization ANCOVA.

Appendix S2. Calculations of jump parameters.