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RESEARCH ARTICLE

Predicting the physiological performance of ectotherms in fluctuating thermal environments

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SUMMARY

Physiological ecologists have long sought to understand the plasticity of organisms in environments that vary widely among years, seasons and even hours. This is now even more important because human-induced climate change is predicted to affect both the mean and variability of the thermal environment. Although environmental change occurs ubiquitously, relatively few researchers have studied the effects of fluctuating environments on the performance of developing organisms. Even fewer have tried to validate a framework for predicting performance in fluctuating environments. Here, we determined whether reaction norms based on performance at constant temperatures (18, 22, 26, 30 and 34°C) could be used to predict embryonic and larval performance of anurans at fluctuating temperatures (18–28°C and 18–34°C). Based on existing theory, we generated hypotheses about the effects of stress and acclimation on the predictability of performance in variable environments. Our empirical models poorly predicted the performance of striped marsh frogs (*Limnodynastes peronii*) at fluctuating temperatures, suggesting that extrapolation from studies conducted under artificial thermal conditions would lead to erroneous conclusions. During the majority of ontogenetic stages, growth and development in variable environments proceeded more rapidly than expected, suggesting that acute exposures to extreme temperatures enable greater performance than do chronic exposures. Consistent with theory, we predicted performance more accurately for the less variable thermal environment. Our results underscore the need to measure physiological performance under naturalistic thermal conditions when testing hypotheses about thermal plasticity or when parameterizing models of life-history evolution.

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Key words: acclimation, development, growth, reaction norm, temperature.

INTRODUCTION

A major goal of ecologists is to understand the phenotypic responses of organisms to environmental variation (for reviews, see Pigliucci, 2001; DeWitt and Scheiner, 2004). In particular, thermal fluctuations have pervasive effects on all levels of biological organization, from biochemical reactions to ecological interactions (Angilletta et al., 2002; Hochachka and Somero, 2002; Jiang and Morin, 2004). Dramatic fluctuations in temperature occur daily in many environments, including ephemeral pools (Johns et al., 1981; Dadour et al., 2001; Niehaus et al., 2006), shallow soils (Shine and Elphick, 2001; Ashmore and Janzen, 2003; Georges et al., 2005) and tidal waters (Stillman and Somero, 2000; Podrabsky and Somero, 2004). Despite the ubiquity of thermal change, studies of development at constant temperatures greatly outnumber studies of development at fluctuating temperatures [for examples of the latter, see the following references (Siddiqui and Barlow, 1972; Qualls and Shine, 1998; Loeschcke et al., 1999; Pétavy et al., 2004; Niehaus et al., 2011a; Niehaus et al., 2011b)]. Instead, impacts of thermal fluctuations are usually inferred from a reaction norm, which describes the relationship between temperature and the phenotype.

Traditionally, thermal reaction norms have been constructed by raising closely related individuals over a range of constant temperatures (see Bubily and Loeschcke, 2002; Olsson and Uller, 2002) (reviewed by Scheiner, 2002). By modelling the effect of temperature on physiological rates, one can arrive at a mathematical function that enables prediction of phenotypes in variable environments. Typically, one integrates the resulting function over time to predict the cumulative physiological performance during a specific period of development (Casagrande et al., 1987; Taylor and Shields, 1990; Worner, 1992; Georges et al., 2005). This approach minimizes the error resulting from Jensen's inequality (Ruel and Ayres, 1999), which tells us that performance in a constant environment does not always equal performance in a variable environment with the same mean temperature. Nevertheless, this approach assumes that chronic exposures to temperature, which one uses to estimate the reaction norm, have the same physiological effects as acute exposures.

Two biological phenomena can generate a mismatch between the predicted and actual performances in fluctuating environments. On the one hand, chronic exposure to an extreme temperature could have a deleterious effect on performance, referred to as thermal stress. If so, reaction norms based on performance at constant temperatures would underestimate performance in a fluctuating environment. On the other hand, chronic exposure could trigger a beneficial response,

referred to as thermal acclimation (e.g. Widdows and Bayne, 1971; Wilson et al., 2007; Condon et al., 2010). In this case, reaction norms based on performance at constant temperatures would cause one to overestimate performance in a fluctuating environment. Understanding the effects of stress and acclimation on models of reactions norms should advance our understanding of phenotypic plasticity in variable environments.

We can draw on evolutionary theory to infer the conditions under which acclimation should cause the greatest disparity between observed and predicted phenotypes. When environments vary among generations, natural selection favours genotypes that can developmentally tune their thermal physiology to match the current environment (Gabriel and Lynch, 1992). The optimal genotype would be capable of specializing to perform at its mean body temperature. If specialization leads to greater performance (Huey and Hertz, 1984; Angilletta et al., 2003), acclimating organisms should experience an increase in performance throughout development. Thus, if acclimation occurs, thermal reaction norms constructed from performance in constant environments might overestimate performance in variable environments. This overestimation should scale proportionally to the intensity of the acclimatory response in constant environments.

In this study, we asked whether reaction norms based on performance in constant environments can predict hatchling, larval and metamorphic performance of the striped marsh frog (*Limnodynastes peronii*, Duméril and Bibron 1841) in fluctuating environments. We also asked whether the ability to predict these phenotypes accords with hypotheses about chronic stress or physiological acclimation. Average water temperatures and the extent of thermal fluctuations can vary considerably among potential breeding sites of these frogs, suggesting that the capacity for thermal acclimation exists within populations. Indeed, previous studies confirmed that thermal sensitivities of locomotor performance acclimate during larval development to constant thermal environments (Wilson and Franklin, 1999). Because constant environments are more likely to promote acclimation (Niehaus et al., 2011a), our ability to predict phenotypes in fluctuating environments should decrease as the magnitude of environmental variation increases (i.e. as the environment of interest differs more from a constant one). Furthermore, the accuracy of our predictions should decrease during ontogeny, reflecting the time course of acclimation. The direction of error (under- *vs* over-prediction) would indicate the relative importance of thermal acclimation *versus* thermal stress at constant temperatures. We directly tested these hypotheses by comparing predicted and observed rates of growth and development in two fluctuating environments. Our results support the hypothesis that thermal stress in constant environments leads to poor predictions of performance in fluctuating environments, and that predictions become less accurate in more variable environments. Our results not only underscore the need to design ecologically relevant treatments in studies of thermal ecology but also might shed some light on widespread patterns of life-history variation in ectotherms.

MATERIALS AND METHODS

Collection and husbandry

We collected eggs from 10 egg masses in southeastern Queensland, Australia, in April 2005. These eggs were immediately transported to the laboratory at the University of Queensland. Based on developmental stages [stages 8–11 (Gosner, 1960)] and field observations, we assumed spawning occurred around 03:00 h on the day of collection and used this time when calculating ages. To minimize the influence of genetic effects on any experimental

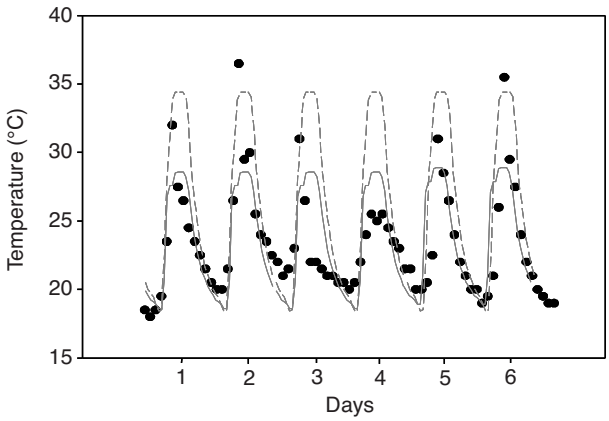

Fig. 1. Thermal variation in nature and in our experiment. Natural temperatures were recorded during the summer breeding season at several field sites in southeastern Queensland during January. Here we present typical daily patterns of temperature for an open shallow pool where tadpoles of *Limnodynastes* spp. were observed (black circles). We generalized these natural cycles to establish two fluctuating treatments in the laboratory: 18–28°C (solid grey line) and 18–34°C (dashed grey line). Mean temperatures of the fluctuating regimes were 22 and 26°C, respectively.

treatment, eggs from all egg masses were mixed together and randomly allocated to thermal treatments. Prior to the experiment, eggs were maintained at 25°C in 10 l plastic containers (100 eggs per container). Animal collection was approved by the Queensland Parks and Wildlife Service, and all experiments were conducted with the authority of the University of Queensland (UQ) Animal Experimentation Ethics Committee. This experiment complied with the current laws of Australia.

Experimental temperatures were assigned based on the maximal daily fluctuations recorded in open habitats in southeastern Queensland during the summer months of December to February using Thermochron data loggers and Dallas Semiconductors (Maxim Integrated Products, Inc., Sunnyvale, CA, USA) (Fig. 1). Tadpoles of this species can be found in both deep and shaded pond environments with limited thermal variability throughout the day, and shallow, exposed ephemeral water bodies that experience marked daily fluctuations (Wilson, 2001; Kraft et al., 2005). In the lab, eggs and tadpoles were housed individually at the following water temperatures: 18, 22, 26, 30 and 34°C, or one of two fluctuating regimes: 18–28°C or 18–34°C. The fluctuating regimes were intended to encompass the range of temperatures observed at our field sites. Temperatures in the water baths (140 l) were determined by ambient temperature (18 and 22°C groups) or were controlled by two aquatic heaters (250–300 W). Water temperatures were maintained within ±0.5°C. Constant circulation of water by aquatic aerators and regular shuffling of containers ensured that no systematic thermal clines occurred within treatments. Heaters in the fluctuating treatments were controlled by electronic timers that turned on at 06:00 h and turned off at 15:30 h, producing naturalistic thermal cycles (Fig. 1). We placed 50 eggs in each thermal treatment and increased or decreased the temperature at a rate of 4–5°C h^{-1}, to prevent extremely rapid change from ambient temperature (25°C) to the experimental temperature. Thus, eggs in the 18–34°C treatment only spent 2 h (rather than 4 h) at 34°C on the first day.

Eggs were individually maintained in ~0.5 ml of water in the wells of 96-well plates. Plates were suspended in water baths and moved around periodically within the bath to ensure uniform thermal profiles among individual eggs. At hatching, larvae were transferred

individually to plastic bottles (1.25 l) for the duration of ontogeny. Each bottle contained a layer of washed gravel (5 cm) and dechlorinated water (700 ml); a mesh cover prevented animals from escaping. Bottles were submerged by 80% in water baths to maintain the desired temperatures. During larval development, tadpoles were fed boiled spinach *ad lib*, and the water in each container was regularly replaced with clean dechlorinated water at the same temperature. At metamorphic climax, most of the water was removed from these containers, leaving the gravel exposed to prevent young animals from drowning. The light cycle (12 h L:12 h D) was similar to that observed in southeastern Queensland during most months of the year.

Development, growth and viability

We monitored individuals every hour up to Gosner stage 25 (yolk absorption) and then every 12–24 h after that. We calculated hourly developmental rates for the period between the estimated time of fertilization and the completion of embryogenesis, based on the inverse of the time to hatching. The total body length of each tadpole (tip of snout to end of tail) was measured using a dissecting microscope (±0.01 mm); this length was divided by the embryonic development time to estimate growth rate.

After hatching, we used the Gosner staging criteria (Gosner, 1960; McDiarmid and Altig, 1999) to categorize the progression toward metamorphic climax, based on morphological and physiological transitions between the fertilized egg (stage 0) and the adult form (stage 46). We recorded the age of all individuals at the following developmental stages: hatching (stage 19–20), stage 25, stage 31, stage 42 and metamorphic climax (stage 46). We defined metamorphic climax (or completion) based on total resorption of the tail. We used certain stages to define periods of development, which we refer to as phases. All developmental rates were calculated as the inverse of age in hours (h^{-1}) between stages.

At most stages, the total body length was measured for each larva using digital calipers (±0.01 mm). However, we did not measure individuals during the period of tail resorption that directly precedes metamorphic climax. Our previous experience with tadpoles of *L. peronii* suggests that weighing larvae can lead to a high mortality, so we only recorded mass after metamorphosis. Growth rates were therefore defined as hourly changes in total body length. Body sizes of metamorphic frogs were obtained within the first 24 h of metamorphosis. Body length was measured with digital calipers (±0.01 mm), and body mass was recorded with a Sartorius balance (±0.01 mg).

Throughout the experiment, we monitored the survival of individuals every 24–48 h. Curves of cumulative survivorship were compared among treatments through a Kaplan–Meier analysis, followed by a Holm–Sidak *post hoc* test.

Statistical models of reaction norms

We used rates of growth and development at constant temperatures to fit statistical models of thermal reaction norms. A separate model was estimated for each developmental phase (embryonic, early-larval, mid-larval and late-larval phases). These models were constrained at thermal extremes to reflect the thermal limit of the frogs' or species' aerobic scope. Upper and lower thermal limits of growth and development were based on previous studies of *L. peronii*. We set the critical thermal minimum equal to 8°C for the growth of larvae, 15°C for the growth of embryos, and 15°C for the development of all stages (R.S.W., unpublished data) (Rogers et al., 2004). The critical thermal maximum for the growth and development of all stages was set equal to 34°C; this temperature not only causes certain mortality during prolonged exposures but also approximates the upper thermal limit of aerobic scope (Niehaus et al., 2011a). These constraints were imposed by augmenting observed data with artificial data at the critical thermal limits; the number of artificial data for the thermal limits equalled the number of real data in each thermal treatment (e.g. before fitting models of larval growth rate, we added 50 observations of 0 mm h^{-1} at 8°C to the observed data).

To determine the best model to describe reaction norms, we compared the fits of various mathematical functions using Akaike's information criterion (AIC) (reviewed by Johnson and Omland, 2004). We compared six functions: quadratic, Gaussian, modified Gaussian, exponentially modified Gaussian, Weibull and beta functions (supplementary material Table S1). Three of these functions – the Gaussian, quadratic and Weibull functions – have been used to theoretically or empirically describe thermal reaction norms (Huey and Kingsolver, 1993; Palaima and Spitze, 2004). The remaining functions were chosen because their complex structure should provide a better fit to non-linear data. We fitted each non-linear model to data with the BFGS method (Broyden, 1970; Fletcher, 1970; Goldfarb, 1970; Shanno, 1970), using the R statistical software package (R Development Core Team, 2007). For each model, we calculated the AIC as follows:

$$\mathrm{AIC} = -2L + 2k + [2k(k+1)/(n-k-1)] , \quad (1)$$

where L equals the log-likelihood estimate of the dependent variable, k equals the number of estimated parameters, and n equals the sample size (Burnham and Anderson, 2002). During each ontogenetic phase, we estimated optimal temperatures for growth and development from the best-fitting model.

Predicting performance in fluctuating environments

We used statistical models of thermal reaction norms to estimate hourly rates of growth and development in the fluctuating thermal treatments. First, we assigned hourly mean temperatures to our two fluctuating treatments (see Fig. 1). Then, we used the best-fitting models at each developmental phase to estimate rates of growth and development. Overall, these rates increased and decreased according to temperature throughout the daily cycle. We used the model of Worner (Worner, 1992) to describe total daily changes in body size (B) and developmental time (D) as:

$$\sum_{t=1}^{N} r(T(t)) , \quad (2)$$

where r equals the incremental rate of growth or development, and T equals the mean temperature at time interval t. We estimated rates of growth and development for each hour of the day and then calculated mean hourly rates over the 24 h period. Predicted rates were regarded as accurate if (i) the mean of the prediction fell within the 95% confidence interval of the measured rate and (ii) the mean of the measured rate fell within the 95% confidence interval of the predicted rate. Confidence intervals of predicted rates were determined from 10,000 non-linear model fits of randomly generated datasets, produced by bootstrapping the empirical data for growth and development at each developmental phase. Specifically, rates in each thermal treatment were sampled with replacement to create new sets of rates with sample sizes equal to those of the experimental groups.

RESULTS

Viability

Embryonic survivorship was high at constant temperatures between 18 and 30°C and in both fluctuating thermal regimes, but no embryos

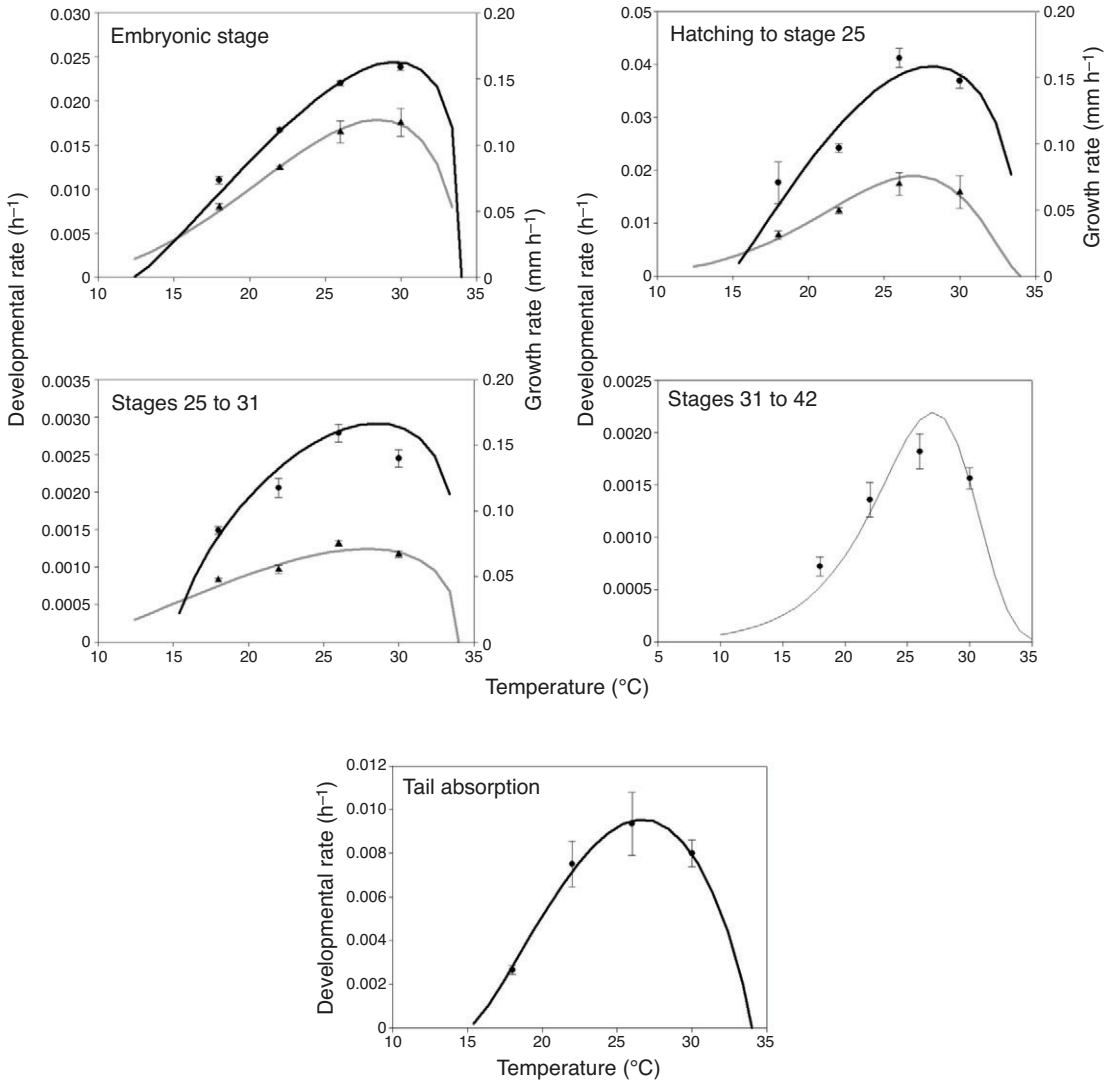

Fig. 2. Thermal sensitivities of growth (black lines, circles) and development rate (grey line, triangles) for: embryonic stage, hatching to stage 25, and stage 25 to stage 31. Thermal sensitivities for development rate only for stage 31 to stage 42 (grey line, circles) and during tail absorption (black line, circles). See Materials and methods for a description of staging criteria. Although regression models were fitted to raw data, means ± 95% confidence intervals are shown for clarity. Model selection criteria are provided in supplementary material Tables S2 and S3.

survived at a constant temperature of 34°C. To determine how chronic exposure to 34°C affected larval viability, we exposed 30 hatchlings to this temperature after they had completed embryogenesis at lower temperatures. Again, no individual survived more than 24 h at 34°C. Thus, we assumed that subsequent stages of larval development would also perish at 34°C. Larvae at fluctuating temperatures experienced the greatest mortality around the time of hatching (median 2–3 days). In contrast, larvae at constant temperatures suffered the greatest mortality at 1 week of age (log rank statistic 43.5, d.f.=5, $P<0.001$). Survivorship over the entire developmental period did not differ among constant or variable treatments (Kaplan–Meier survivorship analysis; $P>0.05$).

Growth and development at constant temperatures

Generally, a beta function best described the thermal sensitivity of growth or development (supplementary material Tables S2–S5 for model rankings and estimated parameters). The only exception to this generality was that a Weibull function better described developmental rate from stages 31 to 42. Based on the best functions, the thermal optimum for developmental rate decreased from 30°C at early stages to 27°C at later stages (Fig. 2; see also supplementary material Table S2). Performance breadths (ranges of temperature at which rates were 80% of the maximum) spanned ~9°C until late stages of development, at which the performance breadth narrowed by several degrees. The thermal optimum (27–28°C) and performance breadths for growth rate did not vary systematically during development (Fig. 2; see also supplementary material Table S3).

Age and size differed among thermal treatments at all stages (supplementary material Table S6), but the patterns of growth and development differed throughout ontogeny (Table 1). Most tadpoles hatched at Gosner stage 19 or 20 (Gosner, 1960), but individuals at higher temperatures reached this stage much earlier. At each larval stage, individuals raised at 18°C were oldest, while those raised at either 26 or 30°C were youngest. Development during the mid-larval stages (25–31) was accelerated for larvae at the cool thermal cycle (18–28°C); consequently, they metamorphosed at about the same age as larvae at 26 and 30°C, even though they took 36% longer

Table 1. Age and size of individuals at ontogenetic stages between hatching and metamorphosis

	Treatment	Age (days)	*N*	95% confidence	Size (mm)	*N*	95% confidence
Hatching	18	3.86	41	0.19	4.85	41	0.14
	22	2.50	46	0.03	4.97	46	0.07
	26	1.90	47	0.03	5.36	44	0.07
	30	1.75	48	0.04	5.35	44	0.08
	18–28	2.39	41	0.04	4.81	41	0.10
	18–34	2.34	48	0.02	5.25	48	0.05
Stage 25	18	6.53	32	0.13	6.92	32	0.16
	22	4.24	25	0.05	7.01	26	0.08
	26	2.94	32	0.03	7.24	31	0.11
	30	2.89	34	0.02	7.01	36	0.12
	18–28	4.50	22	0.11	7.13	25	0.09
	18–34	3.62	33	0.05	7.03	33	0.09
Stage 31	18	33.70	23	0.34	38.07	24	0.63
	22	24.00	11	1.21	32.49	13	0.97
	26	17.50	14	0.61	32.54	20	0.74
	30	19.24	25	0.74	33.44	28	0.83
	18–28	19.87	23	0.64	32.47	20	0.64
	18–34	21.09	23	0.63	31.36	28	0.63
Metamorphosis	18	110.20	25	8.87	33.36	24	1.07
	22	59.08	13	2.13	34.43	14	1.05
	26	44.07	15	1.17	36.06	15	0.62
	30	49.75	28	1.07	34.91	28	0.65
	18–28	48.30	23	1.13	33.51	22	0.53
	18–34	51.96	23	1.13	28.59	22	0.58

Values provided are means.

to reach stage 25. Body lengths at hatching and metamorphosis were longest for larvae at a constant temperature of 26 or 30°C. Although larvae at 18°C were largest at stage 31, they metamorphosed at some of the smallest sizes.

At metamorphosis, body mass differed significantly among treatments (mean square MS=0.21, $F_{5,124}$=25.9, P<0.001). Body masses of metamorphic frogs were very similar among three of the groups raised at constant temperatures (18°C: 0.684±0.054 g; 22°C: 0.653±0.046 g; and 30°C: 0.676±0.031 g), though frogs raised at 26°C were larger (0.736±0.041 g) than those at all other temperatures except for 18°C (Tukey's *post hoc* test; P<0.01). Notably, frogs at 18–28°C were also smaller in mass (0.616±0.028 g; P=0.002) and body length (P=0.009) than those at 26°C (Table 1).

Growth and development at fluctuating temperatures

As in the constant environments, hatching occurred in the fluctuating environments at stages 19 or 20 (Gosner, 1960). Individuals in the two fluctuating environments hatched within a few hours of each other, but body sizes at hatching were markedly larger for hatchlings in the warmer thermal cycle (*t*-test; t_{87}=–8.0, P<0.001). Overall, metamorphic frogs developing at fluctuating temperatures were 10% shorter (*t*-test; t_{123}=–7.72, P<0.0001) and 23% lighter (*t*-test; t_{123}=–7.88, P<0.0001) than frogs at constant temperatures. These differences were largely driven by the small size of individuals emerging from the warm thermal cycle (18–34°C).

Rates of embryonic growth and development were generally under-predicted by reaction norms constructed at constant temperatures (Table 2). At early larval stages (hatching to stage 25), we over-predicted rates of embryonic growth and development at 18–28°C and under-predicted these rates at 18–34°C. At every other stage, rates of growth and development were significantly under-predicted for both fluctuating environments. Only the predicted rate of growth at 18–28°C was indistinguishable from the observed rate. Consistent with one of our hypotheses, our error in predicting growth and development was greater for individuals at 18–34°C than it was for individuals at 18–28°C. Contrary to our other hypothesis, the magnitude of error did not increase steadily throughout ontogeny.

To be sure that our choice of critical thermal limits did not influence our conclusions, we performed a sensitivity analysis. We lowered or raised the critical thermal minimum or critical thermal maximum, respectively, by 4°C and refitted the statistical models described above. For the best-fitting models, the new parameters were used to predict growth and development in the fluctuating environments. We then assessed the direction and significance of the difference between the predicted and observed performance. These outcomes were compared with those for models fitted to different critical thermal limits. For all stages of growth and development except one, the direction and significance of the difference were the same. In the one exception, the predicted development of embryos at 18–34°C was significantly faster when a critical thermal maximum of 38°C was used instead of a critical thermal maximum of 34°C. Nevertheless, our general conclusions about our hypotheses would have been the same for this scenario. Furthermore, tadpoles were unlikely to have developed at temperatures as high as 38°C given that they have no scope for aerobic metabolism at temperatures above 34°C (Niehaus et al., 2011a).

DISCUSSION

A growing number of researchers have recognized the need to understand development under fluctuating temperatures, which better represent the diel cycles of natural environments (e.g. Qualls and Shine, 1998; Dadour et al., 2001; Ashmore and Janzen, 2003; Niehaus et al., 2006; Oufiero and Angilletta, 2006). Still, much of our knowledge about thermal plasticity comes from experiments involving constant temperatures. Studies that have attempted to use data from constant environments to predict outcomes in variable

Table 2. Comparisons of observed and predicted (italics) rates of growth and development in *Limnodynastes peronii* at fluctuating temperatures

Ontogenetic stages	Thermal treatment (°C)	Developmental rate (h^{-1})	% Error	Growth rate (mm h^{-1})	% Error
Embryonic	18–28	0.0175±0.0003 (41)	–3.4	**0.0838±0.0023 (41)**	3.1
		0.0169±0.0002		***0.0864±0.0034***	
	18–34	0.0178±0.0002 (48)	–23.6	0.0934±0.0009 (48)	–29.6
		0.0136±0.0001		*0.0658±0.0026*	
Early larval (hatching to stage 25)	18–28	0.0194±0.0014 (22)	44.8	0.0436±0.0044 (22)	24.1
		0.0281±0.0007		*0.0541±0.0017*	
	18–34	0.0331±0.0013 (33)	–35.0	0.0581±0030 (33)	–35.6
		0.0215±0.0005		*0.0374±0.0013*	
Mid-larval (stages 25 to 31)	18–28	0.0026±0.0001 (26)	–19.2	0.0657±0.0029 (20)	–8.2
		0.0021±0.0001		*0.0603±0.0011*	
	18–34	0.0024±0.0001 (29)	–29.2	0.0586±0.0012 (28)	–20.3
		0.0017±0.0000		*0.0467±0.0008*	
Late larval (stages 31 to 42)	18–28	0.0018±0.0001 (23)	–22.2		
		0.0014±0.0001			
	18–34	0.0016±0.0001 (23)	–43.8		
		0.0009±0.00001			
Tail resorption (stage 42 to metamorphosis)	18–28	0.0077±0.0006 (21)	–11.7		
		0.0068±0.0004			
	18–34	0.0076±0.0006 (21)	–36.8		
		0.0048±0.0003			

Each rate is reported as the mean ± 95% confidence interval, along with the sample size for each observed rate in parentheses. Bold font denotes predicted and observed rates that were statistically indistinguishable.

ones (e.g. Schoolfield et al., 1981; Worner, 1992; Georges et al., 2005) forge an important link between the stable conditions of laboratories and the variable conditions of nature. Nevertheless, predicting performance in variable environments remains challenging because (i) physiological rates relate non-linearly to temperature, and (ii) many organisms can withstand short periods at chronically lethal temperatures (Christian et al., 1986; Tingle and Copland, 1988). The models that most accurately predict phenotypes in variable environments tend to be very complex (van der Have, 2002; Davidson et al., 2003; Lerin, 2004; Georges et al., 2005). Unfortunately, complex models can overfit empirical data, reducing the generality of conclusions and the predictability of patterns (Burnham and Anderson, 2002). Biologists have recently adopted new statistical methods to infer the best models for predicting performance in variable environments (Georges et al., 2005; Angilletta, 2006). We extended this effort by testing *a priori* hypotheses about the predictability of ectotherm performance in variable environments.

We hypothesized that the difference between expected and observed performance stems from either stress or acclimation in constant environments. In other words, prolonged exposure to a constant temperature could change the reaction norm, resulting in either underestimation or overestimation of performance in variable environments. For a scenario of either stress or acclimation, we made two predictions: (1) the magnitude of error would be greater in a more variable environment, and (2) the magnitude of error would increase throughout ontogeny. At most stages, individuals grew and developed more rapidly than we predicted from our models of reaction norms. This result supports the hypothesis that thermal stress at constant temperatures causes substantial error in our predictions. Furthermore, variation in the magnitude of under-prediction enabled us to test our hypotheses. We were able to predict performance in the moderately fluctuating environment more accurately than we could in the highly fluctuating environment. Nevertheless, the predictability of growth and development did not vary systematically throughout ontogeny, contrasting with our second prediction. Below, we discuss some possible explanations for these patterns, which seem to be inconsistent with our hypothesis that acclimation to constant environments explains the mismatch between predicted and observed performance in fluctuating environments.

Reduced performance during chronic exposure to extreme temperatures probably accounts for the underestimation of performance in fluctuating environments. We assumed that growth and development ceased at 34°C because chronic exposure to this temperature leads to certain mortality. Although chronic exposure to high temperatures would prevent growth and development, larvae obviously tolerate acute exposures as evidenced by successful development in an environment that fluctuated between 18 and 34°C. This source of error might be fairly common because researchers studying insects have also under-predicted larval development when relying on reaction norms estimated from development at constant temperatures (Casagrande et al., 1987; Taylor and Shields, 1990; Worner, 1992).

Other mechanisms might contribute to discrepancies between predicted and observed rates of performance. First, both growth and development depend on a plethora of cellular processes that probably differ in their thermal sensitivities (Beck, 1983). In a fluctuating environment, the optimal temperature for each cellular process might be encountered over the course of the day, facilitating performance over longer time scales. In a constant environment, growth or development would proceed more slowly if the temperature were sub-optimal for one or more of the requisite cellular processes. Second, individuals might allocate more resources to growth and development in a fluctuating environment than they do in constant environments. In nature, many organisms shorten developmental periods when conditions deteriorate because of the risk of infection (Warkentin et al., 2001), predation (Wedekind and Muller, 2005) or desiccation (Semlitsch and Wilbur, 1988; Morey and Reznick, 2004). The relatively rapid growth and development of tadpoles in the fluctuating environments could reflect an adaptive response; for

example, fluctuating temperatures can signal the drying of a pool, which would favour genotypes able to accelerate their development (Newman, 1989; Rowe and Ludwig, 1991; Frisch and Santer, 2004; Morey and Reznick, 2004; Rolff et al., 2004).

Life-history theory predicts the reaction norms for age and size at metamorphosis in variable environments (Wilbur and Collins, 1973; Smith-Gill and Berven, 1979; Werner, 1986; Hentschel, 1999; Day and Rowe, 2002; Bruce, 2005; Rudolf and Rödel, 2007). In our experiment, rapid growth at high temperatures was associated with early metamorphosis at a large size. Models that predict this outcome assume that organisms must achieve a minimal size threshold before they can metamorphose or mature (Wilbur and Collins, 1973; Hentschel, 1999; Day and Rowe, 2002). When growth occurs slowly, these models suggest that larvae must delay metamorphosis until reaching such a size threshold. Our results fail to validate this assumption because the average size of metamorphs from the 18°C treatment was larger than that of most of the metamorphs from all other temperature treatments. This result suggests that the metamorphic size at 18°C was greater than the minimal size required for metamorphosis. Interestingly, individuals raised in moderately fluctuating environments (18–28°C) metamorphosed earlier than, and at similar sizes to, individuals raised in a constant environment with approximately the same mean temperature (see Table 1). By contrast, individuals raised in a highly fluctuating environment (18–34°C) metamorphosed later and at smaller sizes than the constant temperature treatment with the same mean of 26°C, suggesting that high temperatures did impair performance to some degree.

The mismatch between rates of performance during chronic and acute exposures to high temperatures has important implications for the evolution of age and size at metamorphosis. Specifically, the optimal reaction norms for these life-history traits depend on the thermal sensitivities of growth rate (Berrigan and Charnov, 1994; Angilletta et al., 2004; Kozlowski et al., 2004). Yet, most models have been evaluated by raising organisms at constant temperatures. If the rate of performance during chronic exposure does not accord with the rate of performance during acute exposure, empirical estimates of thermal sensitivities based on chronically exposed individuals would lead to erroneous conclusions about the optimal reaction norms for age and size at metamorphosis. Therefore, ecologists must endeavour to estimate thermal sensitivities of growth rate through acute exposures to extreme temperatures (Kingsolver and Woods, 1997).

Most of what we know about the thermal plasticity of organisms derives from growth and development measured at constant temperatures, despite the scarcity of such conditions in terrestrial and shallow aquatic environments. As we have shown, rates of growth and development at constant temperatures might poorly reflect these functions under realistic thermal conditions. As we expected, our predictions were less accurate for a highly fluctuating environment than they were for a moderately fluctuating environment. Given the potential for stress or acclimation in constant thermal environments, studies of performance in fluctuating environments should become the norm rather than the exception. Furthermore, models of adaptation in variable environments should focus on multi-dimensional reaction norms, which relate organismal phenotypes to mean temperatures and thermal variances. Such models can indicate whether the study of performance in constant environments will provide accurate information about performance in a variable environment. Both empirical and theoretical attention to this problem would advance our understanding of the complexity of thermal physiology and life history.

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REFERENCES

Angilletta, M. J. (2006). Estimating and comparing thermal performance curves. *J. Therm. Biol.* **31**, 541-545.
Angilletta, M. J., Niewiarowski, P. H. and Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* **27**, 249-268.
Angilletta, M. J., Wilson, R. S., Navas, C. A. and James, R. S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* **18**, 234-240.
Angilletta, M. J., Steury, T. D. and Sears, M. W. (2004). Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integr. Comp. Biol.* **44**, 498-509.
Ashmore, G. M. and Janzen, F. J. (2003). Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia* **134**, 182-188.
Beck, S. D. (1983). Insect thermoperiodism. *Annu. Rev. Entomol.* **28**, 91-108.
Berrigan, D. and Charnov, E. L. (1994). Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. *Oikos* **70**, 474-478.
Broyden, C. G. (1970). The convergence of a class of double-rank minimization algorithms. *J. Inst. Math. Appl.* **6**, 76-90.
Bruce, R. C. (2005). Theory of complex life cycles: application in Plethodontid salamanders. *Herpetol. Monogr.* **19**, 180-207.
Bubily, O. A. and Loeschcke, V. (2002). Effect of low stressful temperature on genetic variation of five quantitative traits in *Drosophila melanogaster. Heredity* **89**, 70-75.
Burnham, K. P. and Anderson, D. R. (2002). *Model Selection and Multimodel Inference: a Practical Information-Theoretic Approach.* New York: Springer.
Casagrande, R. A., Logan, P. A. and Wallner, W. E. (1987). A developmental model for gypsy moth, *Lymantria dispar* (L). *Environ. Entomol.* **16**, 556-562.
Christian, K. A., Tracy, C. R. and Porter, W. P. (1986). The effect of cold exposure during incubation of *Sceloporus undulatus* eggs. *Copeia* **1986**, 1012-1014.
Condon, C. H., Chenoweth, S. F. and Wilson, R. S. (2010). Zebrafish take their cue from temperature but not photoperiod for the seasonal plasticity of thermal performance. *J. Exp. Biol.* **213**, 3705-3709.
Dadour, I. R., Cook, D. F. and Wirth, N. (2001). Rate of development of *Hydrotaea rostrata* under summer and winter (cyclic and constant) temperature regimes. *Med. Vet. Entomol.* **15**, 177-182.
Davidson, G., Phelps, K., Sunderland, K. D., Pell, J. K., Ball, B. V., Shaw, K. E. and Chandler, D. (2003). Study of temperature-growth interactions of entomopathogenic fungi with potential for control of *Varroa destructor* (Acari: Mesostigmata) using a nonlinear model of poikilotherm development. *J. Appl. Microbiol.* **94**, 816-825.
Day, T. and Rowe, L. (2002). Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. *Am. Nat.* **159**, 338-350.
DeWitt, T. J. and Scheiner, S. M. (2004). *Phenotypic Plasticity: Functional and Conceptual Approaches.* Oxford, UK: Oxford University Press.
Fletcher, R. (1970). A new approach to variable metric algorithms. *Comput. J.* **13**, 317-322.
Frisch, D. and Santer, B. (2004). Temperature-induced responses of a permanent-pond and a temporary-pond cyclopoid copepod: a link to habitat predictability? *Evol. Ecol. Res.* **6**, 541-553.
Gabriel, W. and Lynch, M. (1992). The selective advantage of reaction norms for environmental tolerance. *J. Evol. Biol.* **5**, 41-59.
Georges, A., Beggs, K., Young, J. E. and Doody, J. S. (2005). Modelling development of reptile embryos under fluctuating temperature regimes. *Physiol. Biochem. Zool.* **78**, 18-30.
Goldfarb, D. (1970). A family of variable metric updates derived by variational means. *Math. Comp.* **24**, 23-26.
Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183-190.
Hentschel, B. T. (1999). Complex life cycles in a variable environment: predicting when the timing of metamorphosis shifts from resources dependent to developmentally fixed. *Am. Nat.* **154**, 549-558.
Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation.* Princeton, NJ, USA: Princeton University Press.
Huey, R. B. and Hertz, P. E. (1984). Is a jack-of-all-temperatures a master of none? *Evolution* **38**, 441-444.
Huey, R. B. and Kingsolver, J. G. (1993). Evolution of resistance to high temperature in ectotherms. *Am. Nat.* **142**, S21-S46.
Jiang, L. and Morin, P. J. (2004). Temperature-dependent interactions explain unexpected responses to environmental warming in communities of competitors. *J. Anim. Ecol.* **73**, 569-576.
Johns, D. M., Howell, W. H. and Klein-MacPhee, G. (1981). Yolk utilization and growth to yolk-sac absorption in summer flounder (*Paralichthys dentatus*) larvae at constant and cyclic temperatures. *Mar. Biol.* **63**, 301-308.

Johnson, J. B. and Omland, K. S. (2004). Model selection in ecology and evolution. *Trends Ecol. Evol.* **19**, 101-108.
Kingsolver, J. G. and Woods, H. A. (1997). Thermal sensitivity of growth and feeding in *Manduca sexta* caterpillars. *Physiol. Zool.* **70**, 631-638.
Kozlowski, J., Czarnoleski, M. and Dańko, M. (2004). Can optimal resource allocation models explain why ectotherms grow larger in cold? *Integr. Comp. Biol.* **44**, 480-493.
Kraft, P., Wilson, R. S. and Franklin, C. E. (2005). Phenotypic plasticity as a defence strategy in tadpoles of *Limnodynastes peronii*: induction cues, costs and benefits. *Austral. Ecol.* **30**, 558-563.
Lerin, J. (2004). Modeling embryonic development in *Sitona lineatus* (Coleoptera: Curculionidae) in fluctuating temperatures. *Environ. Entomol.* **33**, 107-112.
Loeschcke, V., Bundgaard, J. and Barker, J. S. F. (1999). Reaction norms across and genetic parameters at different temperatures for thorax and wing size traits in *Drosophila aldrichi* and *D. buzzatii*. *J. Evol. Biol.* **12**, 605-623.
McDiarmid, R. W. and Altig, R. (1999). *Tadpoles: The Biology of Anuran Larvae*, pp. 10-11. Chicago, IL, USA: The University of Chicago Press.
Morey, S. R. and Reznick, D. N. (2004). The relationship between habitat permanence and larval development in California spadefoot toads: field and laboratory comparisons of developmental plasticity. *Oikos* **104**, 172-190.
Newman, R. A. (1989). Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. *Ecology* **70**, 1775-1787.
Niehaus, A., Wilson, R. S. and Franklin, C. E. (2006). Short- and long-term consequences of thermal variation in the larval environment of anurans. *J. Anim. Ecol.* **75**, 686-692.
Niehaus, A., Wilson, R. S., Seebacher, F. and Franklin, C. E. (2011a). Striped marsh frog (*Linnodynastes peronii*) tadpoles do not acclimate metabolic performance to thermal variability. *J. Exp. Biol.* **214**, 1965-1970.
Niehaus, A. C., Wilson, R. S., Storm, J. J. and Angilletta, M. J. (2011b). Fall field crickets did not acclimate to simulated seasonal changes in temperature. *J. Comp. Physiol. B.* doi: 10.1007/s00360-011-0611-1.
Olsson, M. and Uller, T. (2002). Developmental stability and genetic architecture: a comparison within and across thermal regimes in tadpoles. *J. Evol. Biol.* **15**, 625-633.
Oufiero, C. E. and Angilletta, M. J. (2006). Convergent evolution of embryonic growth and development in the eastern fence lizard (*Sceloporus undulatus*). *Evolution* **60**, 1066-1075.
Palaima, A. and Spitze, K. (2004). Is a jack-of-all-temperatures a master of none? An experimental test with *Daphnia pulicaria* (Crustacea: Cladocera). *Evol. Ecol. Res.* **6**, 215-225.
Pétavy, G., David, J. R., Debat, V., Gibert, P. and Moreteau, B. (2004). Specific effects of cycling stressful temperatures upon phenotypic and genetic variability of size traits in *Drosophila melanogaster*. *Evol. Ecol. Res.* **6**, 873-890.
Pigliucci, M. (2001). *Phenotypic Plasticity: Beyond Nature and Nurture.* Baltimore, MD, USA: Johns Hopkins University Press.
Podrabsky, J. E. and Somero, G. N. (2004). Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. *J. Exp. Biol.* **207**, 2237-2254.
Qualls, F. J. and Shine, R. (1998). Geographic variation in lizard phenotypes: importance of the incubation environment. *Biol. J. Linn. Soc. Lond.* **64**, 477-491.
R Development Core Team (2007). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
Rogers, K. D., Seebacher, F. and Thompson, M. B. (2004). Biochemical acclimation of metabolic enzymes in response to lowered temperature in tadpoles of *Limnodynastes peronii*. *Comp. Biochem. Physiol.* **137A**, 731-738.
Rolff, J., Van de Meutter, F. and Stoks, R. (2004). Time constraints decouple age and size at maturity and physiological traits. *Am. Nat.* **164**, 559-564.
Rowe, L. and Ludwig, D. (1991). Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology* **72**, 413-427.
Rudolf, V. H. W. and Rödel, M. O. (2007). Phenotypic plasticity and optimal timing of metamorphosis under uncertain time constraints. *Evol. Ecol.* **21**, 121-142.
Ruel, J. J. and Ayres, M. P. (1999). Jensen's inequality predicts effects of environmental variation. *Trends Ecol. Evol.* **14**, 361-366.
Scheiner, S. M. (2002). Selection experiments and the study of phenotypic plasticity. *J. Evol. Biol.* **15**, 889-898.
Schoolfield, R. M., Sharpe, P. J. H. and Magnuson, C. E. (1981). Non-linear regression of biological temperature-dependent rate models based on absolute reaction-rate theory. *J. Theor. Biol.* **88**,719-731.
Semlitsch, R. D. and Wilbur, H. M. (1988). Effects of pond drying time on metamorphosis and survival in the salamander *Ambystoma talpoideum*. *Copeia* **1988**, 978-983.
Shanno, D. F. (1970). Conditioning of quasi-Newton methods for function minimization. *Math. Comp.* **24**, 647-656.
Shine, R. and Elphick, M. J. (2001). The effect of short-term weather fluctuations on temperatures inside lizard nests, and on the phenotypic traits of hatchling lizards. *Biol. J. Linn. Soc.* **72**, 555-565.
Siddiqui, W. H. and Barlow, C. A. (1972). Population growth of *Drosophila melanogaster* at constant and alternating temperatures. *Ann. Entomol. Soc. Am.* **65**, 993-1001.
Smith-Gill, S. J. and Berven, K. A. (1979). Predicting amphibian metamorphosis. *Am. Nat.* **113**, 563-585.
Stillman, J. H. and Somero, G. N. (2000). A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiol. Biochem. Zool.* **73**, 200-208.
Taylor, P. S. and Shields, E. J. (1990). Development of the armyworm (Lepidoptera: Noctuidae) under fluctuating daily temperature regimes. *Environ. Entomol.* **19**, 1422-1431.
Tingle, C. C. D. and Copland, M. J. W. (1988). Predicting development of the mealybug parasitoids *Anagyrus pseudococci*, *Leptomastix dactylopii* and *Leptomastidae abnormis* under glasshouse conditions. *Entomol. Exp. Appl.* **46**, 19-28.
van der Have, T. M. (2002). A proximate model for thermal tolerance in ectotherms. *Oikos* **98**, 141-155.
Warkentin, K. M., Currie, C. R. and Rehner, S. A. (2001). Egg-killing fungus induces early hatching of red-eyed treefrog eggs. *Ecology* **82**, 2860-2869.
Wedekind, C. and Muller, R. (2005). Risk-induced early hatching in salmonids. *Ecology* **86**, 2525-2529.
Werner, E. E. (1986). Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am. Nat.* **128**, 319-341.
Widdows, J. and Bayne, B. L. (1971). Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J. Mar. Biolog. Assoc. UK* **51**, 827-843.
Wilbur, H. M. and Collins, J. P. (1973). Ecological aspects of amphibian metamorphosis. *Science* **182**, 1305-1314.
Wilson, R. S. (2001). Geographic variation in thermal sensitivity of jumping performance in the frog *Limnodynastes peronii*. *J. Exp. Biol.* **24**, 4227-4236.
Wilson, R. S. and Franklin, C. E. (1999). Thermal acclimation of locomotor performance in tadpoles of the frog *Limnodynastes peronii*. *J. Comp. Physiol. B* **169**, 445-451.
Wilson, R. S., Hammill, E. and Johnston, I. A. (2007). Competition moderates the benefits of thermal acclimation to reproductive performance in male eastern mosquitofish. *Proc. R. Soc. Lond. B* **274**, 1199-1204.
Worner, S. P. (1992). Performance of phonological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. *Environ. Entomol.* **21**, 689-699.