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RESEARCH ARTICLE

Temperature and UV-B-insensitive performance in tadpoles of the ornate burrowing frog: an ephemeral pond specialist

Pippa Kern*, Rebecca L. Cramp and Craig E. Franklin

ABSTRACT

Animals may overcome the challenges of temperature instability through behavioural and physiological mechanisms in response to short- and long-term temperature changes. When ectotherms face the challenge of large diel temperature fluctuations, one strategy may be to reduce the thermal sensitivity of key traits in order to maintain performance across the range of temperatures experienced. Additional stressors may limit the ability of animals to respond to these thermally challenging environments through changes to energy partitioning or interactive effects. Ornate burrowing frog (*Platyplectrum ornatum*) tadpoles develop in shallow ephemeral pools that experience high diel thermal variability (>20°C) and can be exposed to high levels of UV-B radiation. Here, we investigated how development in fluctuating versus stable temperature conditions in the presence of high or low UV-B radiation influences thermal tolerance and thermal sensitivity of performance traits of *P. ornatum* tadpoles. Tadpoles developed in either stable (24°C) or fluctuating temperatures (18–32°C) under high or low UV-B conditions. Tadpoles were tested for upper critical thermal limits, thermal dependence of resting metabolic rate and maximum burst swimming performance. We hypothesised that developmental responses to thermal fluctuations would increase thermal tolerance and reduce thermal dependence of physiological traits, and that trade-offs in the allocation of metabolic resources towards repairing UV-B-induced damage may limit the ability to maintain performance over the full range of temperatures experienced. We found that *P. ornatum* tadpoles were thermally insensitive for both burst swimming performance, across the range of temperatures tested, and resting metabolic rate at high temperatures independent of developmental conditions. Maintenance of performance led to a trade-off for growth under fluctuating temperatures and UV-B exposure. Temperature treatment and UV-B exposure had an interactive effect on upper critical thermal limits possibly due to the upregulation of the cellular stress response. Thermal independence of key traits may allow *P. ornatum* tadpoles to maintain performance in the thermal variability inherent in their environment.

KEY WORDS: Thermal fluctuation, Thermal insensitivity, Thermal tolerance, Metabolic rate

INTRODUCTION

Thermal variability can impact upon the fitness of ectotherms through effects on growth, reproduction and whole-animal performance as a consequence of the thermal sensitivity of underlying physiological processes (Huey and Stevenson, 1979; Huey, 1982). Because of the non-linear response of metabolic rate to temperature change, fluctuating temperatures can increase the energy demand required for cell maintenance at higher temperatures. During development, fluctuating temperatures can lead to increased rates of development and a reduction in body size (Dong et al., 2006; Niehaus et al., 2006; Dhillon and Fox, 2007; Du and Shine, 2010) compared with that of animals developing at the equivalent mean temperature (Ruel and Ayres, 1999; Williams et al., 2012). As such, animals may employ behavioural and/or physiological strategies to mitigate the challenges associated with fluctuating environmental temperatures. Thermally variable conditions lead to increased thermal tolerance in some adult aquatic ectotherms (Feldmeth et al., 1974; Widdows, 1976; Sinclair et al., 2006), which suggests that exposure to thermally variable environments can improve performance breadth in some cases. It has yet to be established how variable conditions affect the thermal dependence of performance traits and the physiological mechanisms that underpin them (but see Niehaus et al., 2011). Responses that increase thermal tolerance and reduce the thermal dependence of physiological processes and performance traits may buffer animals from the costs associated with thermally variable environments (Schaefer and Ryan, 2006; Williams et al., 2012) and allow them to remain active across the range of temperatures experienced.

The large diel thermal variability characteristic of some aquatic environments (i.e. tidal pools and ephemeral pools) can be due to solar heating of such exposed environments. Animals inhabiting these environments are therefore exposed to high levels of ultraviolet-B (UV-B) radiation. UV-B radiation causes cellular damage through the absorption of radiation by DNA, which must be repaired to limit disruption to DNA transcription (Friedburg et al., 2006). The consequences of UV-B exposure during development include reduced growth rates (Tevini, 1993; Caldwell et al., 1998), reduced locomotor ability (Álvarez and Nicieza, 2002), reduced metabolic scope (Ylonen et al., 2004) and increased time to metamorphosis in tadpoles (Belden et al., 2000; Pahkala et al., 2001; Belden and Blaustein, 2002; Pahkala et al., 2003). These effects indicate energy allocation to repair UV-B-induced DNA damage (Alton et al., 2012). Increased maintenance costs and energy allocation during UV-B exposure may divert energy from compensatory mechanisms to deal with thermal variability in order to meet energy requirements for growth and development.

Interactive effects of temperature and UV-B radiation may influence the thermal tolerance and thermal sensitivity of performance traits. UV-B radiation interacts with environmental factors such as pH, temperature and predator cues (Pahkala et al., 2002; van Uitregt et al., 2007; Alton et al., 2010) to produce synergistic effects on animal mortality rates. UV-B radiation has been shown to have temperature-dependent effects on growth, survival and thermal tolerance (Winckler and Fidhiany, 1996;

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Winckler and Fidhiany, 1999; van Uitregt et al., 2007), but how these factors interact to influence physiological responses to thermal variability has not been considered. Investigating these types of interactions will broaden our knowledge of the influence of temperature and other environmental factors on the thermal sensitivity of performance traits (Clusella-Trullas et al., 2011).

The challenge of dealing with large diel thermal variation and high UV-B radiation is pertinent for the larvae of amphibian species that breed in ephemeral pools of arid and semi-arid zones. Species such as the Australian ornate burrowing frog (*Platyplectrum ornatum*, Gray 1841) breed after heavy rain in ephemeral water bodies, including small puddles, and development is rapid (21 days) (Barker et al., 1995) to accommodate a short hydroperiod. Breeding pools are often exposed and can experience considerable diel thermal variation (>20°C; Fig. 1) and high UV-B radiation (P.K., unpublished observation) associated with the solar warming of their ephemeral habitats (Blaustein et al., 2001). Furthermore, high metabolic demands for growth during development may limit metabolic scope (Killen et al., 2007; Rombough, 2011), and may reduce the energy available for physiological mechanisms to buffer performance from thermal variability and the effects UV-B in the environment. As such, *P. ornatum* tadpoles provide a good system to investigate interactive effects between thermal variability and UV-B radiation on thermal tolerance and the thermal sensitivity of performance traits, and will allow for a greater understanding of how animals allocate resources during development in these environments.

In this study, we aimed to determine physiological responses of *P. ornatum* tadpoles to the thermal variability and UV-B exposure inherent in their environment. To assess this, we examined the interactive influence of UV-B radiation and fluctuating temperatures on thermal tolerance and the thermal sensitivity of performance traits. We examined resting metabolic rate in combination with burst swimming performance, an important predator-avoidance mechanism for tadpoles (Wilson and Franklin, 1999), to determine whether key fitness traits were buffered from environmental thermal variability through reduced thermal sensitivity. We hypothesised that developmental responses to thermal fluctuations would reduce thermal dependence of physiological traits, and that interactive effects between fluctuating temperatures and UV-B radiation may reduce the ability of organisms to maintain these performance traits over the full range of temperatures experienced.

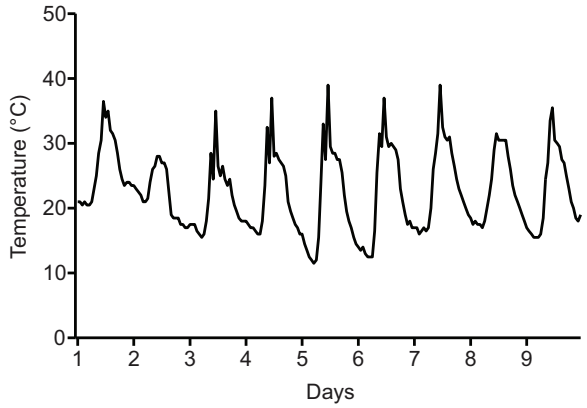

Fig. 1. Thermal variability of *Platyplectrum ornatum* developmental environment. Temperature profile from a data logger (February 2012) positioned in an ephemeral pool near Dalby (Queensland, Australia), where foam nests of *P. ornatum* were collected.

RESULTS

Survival, development time and body condition

For survival, there was a significant interaction between treatment temperature and UV-B treatment ($F_{1,28}$=5.12, P=0.03). Survival was unaffected by high (UV+) or low (UV–) UV-B for tadpoles in the 24°C treatment (24°C/UV+=41.3±2.72.5%; 24°C/UV–=38.8±2.2%), whereas for tadpoles in the 18–32°C temperature treatment, high UV-B reduced survival (18–32°C/UV+=35.0±4.6%; 18–32°C/UV–=46.7±2.4%). The average time for tadpoles to reach development stage 35–37 (Gosner, 1960) was 39±1 days with no difference in development time between tadpoles from temperature ($F_{1,374}$=0.44, P=0.51) and UV-B treatments ($F_{1,373}$=0.37, P=0.36). At stage 35–37, body mass and body and tail length were significantly different between treatments. The body mass of tadpoles was significantly affected by both temperature treatment ($F_{1,374}$=22.25, P<0.001) and UV-B treatment ($F_{1,373}$=4.08, P=0.04; Fig. 2) independently, with no interactive effect ($F_{1,372}$=0.61, P=0.61). Temperature treatment had the greatest influence on body mass with tadpoles from the thermally stable treatments (24°C/UV+, N=94, M_b=0.27±0.01 g; 24°C/UV–, N=91, M_b =0.28±0.01 g) being heavier than tadpoles from thermally fluctuating treatments (18–32°C/UV+, N=84, M_b=0.24±0.01 g; 18–32°C/UV–, N=107, M_b=0.25±0.01 g). Within temperature treatments, the exposure to high UV-B reduced body mass, with tadpoles in the UV+ treatment being significantly lighter than those in the UV– treatment within the same temperature regime. Temperature treatment also significantly affected body length ($F_{1,368}$=15.35, P<0.001; 24°C/UV+, N=91, BL=12.18±0.09 mm; 24°C/UV–, N=90, BL=12.21±0.09 mm; 18–32°C/UV+, N=83, BL=11.82±0.10 mm; 18–32°C/UV–, N=106, BL=11.74±0.10 mm), tail length ($F_{1,368}$=7.77, P=0.006; 24°C/UV+, tail length=16.10±0.14 mm; 24°C/UV–, tail length=16.0±0.13 mm; 18–32°C/UV+, tail length=16.05±0.14 mm; 18–32°C/UV–, tail length=16.69±0.17 mm) and total length ($F_{1,368}$=14.11, P<0.001; 24°C/UV+, TL=28.0±0.21 mm; 24°C/UV–, TL=27.74±0.19 mm; 18–32°C/UV+, TL=28.38±0.21 mm; 18–32°C/UV–, TL=28.9±0.23 mm) of tadpoles, with tadpoles in 18–32°C treatments being shorter in each measure than tadpoles that developed at a constant 24°C.

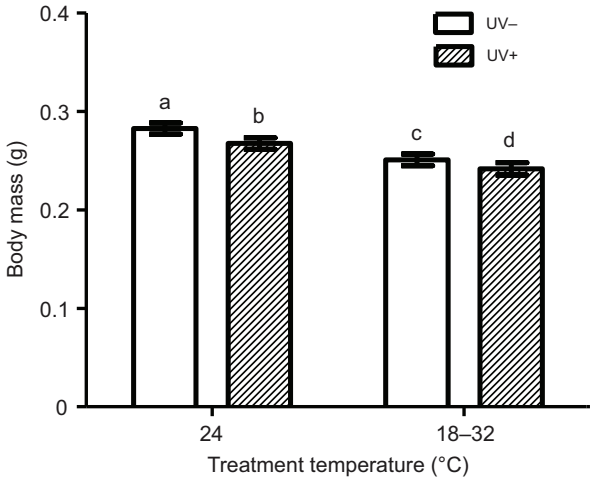

Fig. 2. The effects of fluctuating temperature and UV-B radiation on body mass of *P. ornatum* tadpoles. Both fluctuating temperature ($F_{1,374}$=22.25, P<0.001) and the presence of UV-B ($F_{1,373}$=4.08, P=0.04) significantly reduced body mass. 24°C/UV+, N=94; 24°C/UV–, N=91; 18–32°C/UV+, N=84; 18–32°C/UV+, N=107. Different letters denote significant differences.

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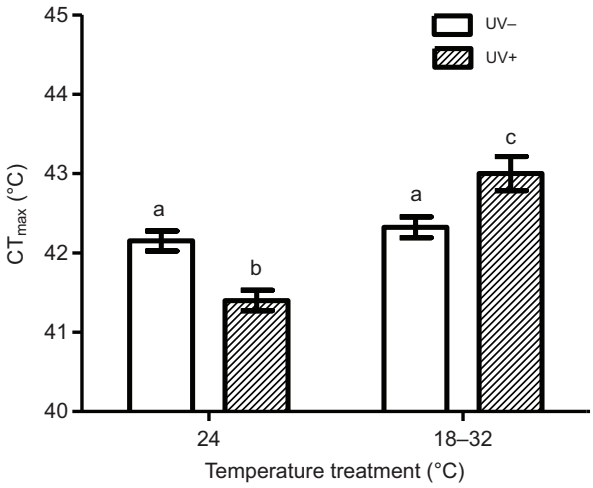

Fig. 3. The interactive effects of temperature treatment and UV-B radiation on CT_{max} of *P. ornatum* tadpoles. There was a significant interaction between temperature treatment and UV-B treatment ($F_{1,28}$=22.91, P<0.001) for CT_{max}. N=8 for all treatments. Different letters denote significant differences.

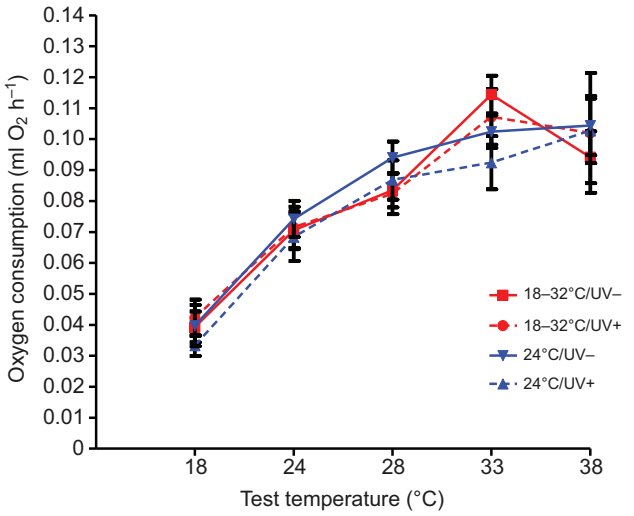

Fig. 4. The thermal dependence curves of RMR for *P. ornatum* tadpoles in thermally stable or thermally variable treatments, with or without UV-B radiation. Oxygen consumption was not significantly different between tadpoles from the fluctuating temperature treatment and those in stable temperatures. UV-B exposure had no effect on oxygen consumption. Oxygen consumption increased with test temperature for the lower range of temperatures tested ($F_{1,167}$=306.13, P<0.001).

UV-B treatment had no effect on body length parameters (BL, $F_{1,367}$=0.25, P=0.62; tail length, $F_{1,367}$=1.35, P=0.25; TL, $F_{1,367}$=0.36, P=0.55).

Critical thermal maximum (CT_{max})

There was a significant interaction (N=8, $F_{1,28}$=22.91, P<0.001) between treatment temperature and UV-B treatment, which influenced CT_{max} (Fig. 3). The CT_{max} of tadpoles from the two thermal treatments was not significantly different when exposed to low levels of UV-B (UV–). Exposure to high levels of UV-B (UV+) caused a 0.84°C reduction in CT_{max} for tadpoles in the stable thermal treatment and a 0.76°C increase in CT_{max} for tadpoles in the fluctuating thermal treatment.

Resting metabolic rate (RMR)

There was no effect of rearing temperature or UV-B treatment on RMR (Fig. 4, Table 1). Test temperature itself significantly affected oxygen consumption (N=7–11 for all test temperatures and treatments, $F_{1,167}$=306.13, P<0.001) with oxygen consumption generally increasing with temperature, although oxygen consumption at 38°C was not significantly different from oxygen consumption at 28 or 33°C (Bonferroni adjusted P>0.05). The Q_{10} for RMR showed reduced thermal sensitivity at the higher temperatures where Q_{10} (28–38°C) ranged between 1.11 and 1.24 compared with lower temperatures where Q_{10} (18–28°C) ranged from 1.95 to 2.61 (Table 2).

Burst swimming performance

There was no effect of rearing temperature or UV-B treatment on maximum burst swimming performance (Fig. 5, Table 3). Test temperature significantly affected burst swimming speed (N=7–12 for all treatments and test temperatures, $F_{1,160}$=7.72, P=0.006) although this difference only lay between the 18 and 33°C test temperatures (Bonferroni adjusted P=0.009). Burst swimming performance at all other test temperatures was not significantly different. Q_{10} (18–38°C) values for maximum burst swimming performance ranged between 1.1 and 1.2 over the entire range of temperatures, indicating low thermal sensitivity (Table 4).

DISCUSSION

Ornate burrowing frog tadpoles demonstrated innate thermal insensitivity of burst swimming performance and metabolism (at high temperatures) and the allocation of resources to maintain key traits, which reflects the thermal variability of their thermal environment. We predicted that tadpoles would increase thermal tolerance and reduce the thermal dependence of RMR and burst swimming performance in response to fluctuating temperatures, and that the interaction with UV-B radiation may reduce this response to fluctuating temperatures. This was not observed; rather, tadpoles maintained performance independently of environmental temperature and UV-B treatment. Our results suggest that: (1) thermal dependence of RMR and burst swimming performance was unaffected by thermal fluctuations or UV-B exposure in *P. ornatum* tadpoles, and (2) ornate burrowing frog tadpoles appear to have innate thermal insensitivity, which may allow them to maintain burst swimming performance and physiological function in a thermally challenging environment.

Metabolism, which underlies important fitness traits by providing energy for growth, development and performance (Angilletta et al., 2006; Wilson et al., 2007; Seebacher, 2009), is a thermally dependent process. In the absence of mechanisms to reduce the thermal sensitivity of metabolism, fitness may be reduced as a result of variable energy availability. Tadpoles reared in thermally variable conditions retain the same thermal sensitivity of metabolic rate as animals reared in thermally stable conditions. All tadpoles showed reduced thermal sensitivity of metabolism between 28 and 38°C. While tadpoles cannot avoid the direct effect of temperature on metabolism across the range of temperatures experienced, reduced thermal sensitivity at higher temperatures may buffer tadpoles from high metabolic demands during peak environmental temperatures.

Although metabolic rate was not different between any treatment, temperature effects on metabolism during the warm part of thermal oscillations may disproportionately increase metabolic demand beyond what would be predicted by the mean temperature (Williams et al., 2012). Both temperature and UV-B exposure affected the body

Table 1. ANOVA table from non-linear regression on RMR data

	a			*b*			*c*		
	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value
Temperature	1,157	0	0.99	1,157	1.03	0.31	1,157	0.01	0.92
UV	1,157	0.326	0.57	1,157	1.17	0.28	1,157	0.90	0.35
Body mass	1,157	285.41	<.0001	1,157	0.10	0.76	1,157	0.73	0.39
Temperature × UV interaction	1,157	0.37	0.55	1,157	0.80	0.37	1,157	0.11	0.74

RMR, resting metabolic rate.
Data are for parameters from the function: $y=ax^2+bx+c$.

condition of tadpoles. For tadpoles in the fluctuating temperature treatment, it appears that high temperatures created a mismatch between metabolic demand and available energy, resulting in a reduction of body mass and length. Exposure to high UV-B exaggerated the reduction in body size, above and beyond the effects of reduced density due to high mortality for tadpoles exposed to fluctuating temperatures and high UV-B levels.

Burst swimming performance was almost entirely thermally insensitive, suggesting inherent temperature independence for this trait. Burst swimming performance is a key fitness trait for tadpoles, which depend extensively upon it for surviving predation and for foraging (Watkins, 1996; Wilson and Franklin, 1999). Ornate burrowing frog tadpoles appear to overcome temperature effects on burst swimming performance, which may allow them to maintain predator avoidance mechanisms in environments characterised by thermal variability. Compared with related anuran species, ornate burrowing frog tadpoles had far lower Q_{10} values for burst swimming performance (Wilson and Franklin, 1999; Niehaus et al., 2011). Our results indicate that *P. ornatum* tadpoles are able to buffer burst swimming performance from the effects of thermal perturbation and that this response occurs independently of developmental conditions. Maintenance of burst swimming performance across a range of temperatures reflects the inherent thermal variability of their developmental environment.

Some killifish also employ this strategy to deal with a similar range of environmental temperatures (Fangue et al., 2008). However, metabolic rate in killifish reflects the thermal independence of their swimming performance, which allows energy requirements for maintaining constant performance to be met. For *P. ornatum* tadpoles, varying energy availability due to thermodynamic effects on metabolism imposed no constraints on performance over the temperature range tested. The energy required to maintain performance, especially at low temperatures, must represent a large portion of the energy budget of these tadpoles during development, and may reduce energy available for other traits such as growth.

Reallocation of energy from growth to key traits may improve chances of survival. Such compensatory partitioning occurs when stressors impose additional metabolic costs and the energy required to support one trait is supplied by reducing resources available for some other function (Wieser, 1989; Rombough, 2011). Unexpectedly, development time was not influenced by developmental conditions. Fluctuating temperatures have led to shorter larval periods in other amphibians (Niehaus et al., 2006; Měráková and Gvoždík, 2009) and other groups of animals (Shine et al., 1997; Du and Shine, 2010). For tadpoles, increasing development rates allow individuals to escape deteriorating conditions. Our results indicate that although resources are diverted from growth to deal with imposed conditions, the development time of this species was maintained. Perhaps the short development time already represents the minimum possible for this species, which allows them to reproduce in highly temporal breeding habitats. This species appears to allocate resources to key survival traits that allow it to survive in a metabolically costly larval environment.

Surprisingly, temperature treatment alone did not influence the upper thermal limit of tadpoles. This contrasts with the results of numerous studies where thermal fluctuations increased upper thermal limits (Feldmeth et al., 1974; Otto, 1974; Schaefer and Ryan, 2006) close to what would be expected with acclimation to the peak temperature of the fluctuation (Otto, 1974). Extension of thermal tolerance breadth may only occur when the peak of the oscillation is above the thermal optimum of the species (Woiwode and Adelman, 1992), as the upper temperature experienced is likely to be the cue for extending CT_{max} (Heath, 1963). In this study, the temperature at which performance was optimised was ~33°C (Fig. 5), which lies just above the upper peak of the temperature oscillations of the fluctuating treatment group. Therefore, the upper temperature of the fluctuating temperature treatment may not have been high enough to induce an increase in thermal tolerance.

UV-B exposure had variable effects on CT_{max} depending on temperature treatment. Exposure to high UV-B radiation resulted in an increase in CT_{max} for tadpoles in the fluctuating temperature treatment, and a decrease in CT_{max} for tadpoles held at stable temperatures. Exposure to high levels of UV-B radiation resulted in reduced body mass of tadpoles. Alton et al. (Alton et al., 2012) showed that UV-B exposure is energetically costly for tadpoles. The reduced body mass of tadpoles exposed to UV-B may therefore be indicative of these costs with a reduction in energy allocated to growth. Repair of UV-B-induced damage and a likely upregulation of the cell stress response (CSR) may account for some of the costs of UV-B exposure. The CSR plays an important role in preventing and repairing macromolecular damage involving heat shock proteins (Kültz, 2003; Kültz, 2005). There are strong indications that the induction of heat shock proteins plays a direct or indirect role in determining thermal tolerance (i.e. CT_{max}) (Fangue et al., 2011; Tomanek, 2008). Pyrimidine diamers that form when UV radiation damages DNA act as a signal to induce the CSR (Mitchel and Morrison, 1984; Anderson et al., 1988). Short UV wavelengths have

Table 2. Q_{10} values for RMR for *P. ornatum* tadpoles in thermally stable or thermally variable treatments, with or without UV-B radiation

	18–32°C/UV–	18–32°C/UV+	24°C/UV–	24°C/UV+
Q_{10} (18–38°C)	1.55	1.55	1.76	1.62
Q_{10} (18–28°C)	1.95	2.12	2.61	2.36
Q_{10} (28–38°C)	1.24	1.13	1.18	1.11

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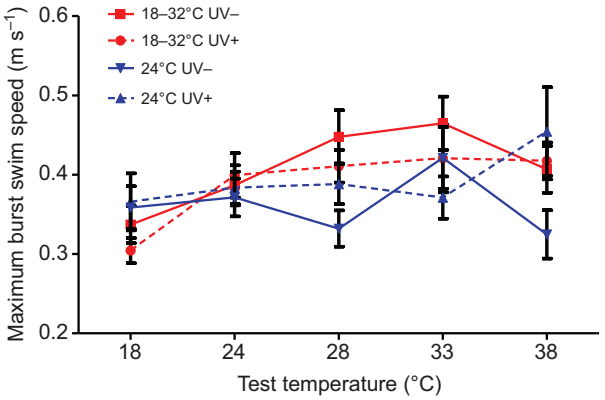

Fig. 5. The interactive effects of thermal variability and UV-B radiation on the thermal dependence of burst swimming speed in *P. ornatum* tadpoles. Swimming performance was not significantly affected by temperature or UV-B treatment. Swimming performance was found to be thermally insensitive, with burst swimming performance only significantly different ($F_{1,160}$=7.72, P=0.006) between 18 and 33°C (Bonferroni adjusted P=0.009).

been shown to induce heat shock proteins (Mitchel and Morrison, 1984; Anderson et al., 1988). It is possible that tadpoles exposed to UV-B in the fluctuating thermal treatment may have incidentally increased CT_{max} through induction of the CSR as a reaction to UV-B-induced damage. The mechanisms that lead to a reduction in CT_{max} for tadpoles in constant temperature were not established although previously recognised interactive effects of UV-B radiation and temperature (van Uitregt et al., 2007) may be important in explaining the reduction of thermotolerance. This interactive effect of temperature and UV-B radiation on upper thermal limits may have important implications for survival in a warming climate (Duarte et al., 2012).

Evidently, for this species, some physiological processes are buffered from environmental variations during development, as the same phenotype for these traits was produced regardless of developmental conditions. This environmental canalisation, which refers to the insensitivity of a trait to environmental factors (Waddington, 1942; Wagner et al., 1997; Debat and David, 2001), protects these traits against environmental perturbation. Canalisation of traits is thought to occur under natural selection and indicates the importance of such traits to fitness as they are maintained independently of environmental conditions (Waddington, 1942; Stearns and Kawecki, 1994).

Our results suggest that canalisation of traits may buffer development from the unpredictable environmental conditions that *P. ornatum* tadpoles experience *in situ*. This species shows inherent capacities to deal with some challenges of diel thermal variation through the thermal independence of burst swimming performance and metabolism at high temperatures, the allocation of resources to maintain key traits and a rapid development time. These traits reflect ecological conditions and may allow ornate burrowing frog tadpoles to maintain performance and physiological function over the range of environmental temperatures they experience. While thermal independence of key traits makes these tadpoles robust in dealing with some challenges of thermal variability inherent in their ephemeral environments, canalisation of traits may reduce their ability to respond to increased variability predicted from current climate models. Increased temperatures and temperature variability may cause ephemeral breeding pools to evaporate more quickly. If tadpoles do not have the capacity to increase development rate they may not be able to reach metamorphosis before habitats dry. Furthermore, failure to mitigate energetic costs of fluctuating temperatures may push metabolic demands beyond the capacity of individuals.

MATERIALS AND METHODS

Egg masses of *P. ornatum* were collected from flooded roadsides in known breeding habitats near Dalby, Queensland, Australia. Egg masses were kept at ~24°C overnight before being transported to The University of Queensland. Once free swimming (1 day after collection), tadpoles were divided into 1 l replicate containers (eight per treatment) at an initial density of 30 tadpoles l^{-1}. Replicates were maintained in 35 l waterbaths to ensure uniform temperature conditions within each treatment. Tadpoles were introduced to temperature treatments in the evening when temperature cycles reached 24°C (Fig. 6). UV-B exposure commenced the following morning. Tadpoles where fed boiled spinach once a day, with excess food removed and water quality checked daily and changed as needed.

Tadpoles were exposed to a factorial combination of thermal variability and UV-B radiation: stable temperature (24±1°C) or fluctuating temperatures (18–32°C with a mean of 24±1°C) and high (UV+) or low (UV–) UV-B exposure (Fig. 6). Cycling water temperatures were maintained with aquarium heaters (55 W, Aqua One) submerged in the surrounding waterbath, connected to electronic timers. Aquarium water pumps were used to create uniform temperatures across replicate containers. UV-B radiation was generated using 40 W linear fluorescent bulbs (Repti-Glo Exo Tera, Montreal, Canada). Larvae in the UV+ treatments were exposed to 4.7±1.5 µW cm^{-2} of UV-B for 13 h per day with an additional peak of 36±4 µW cm^{-2} for 5 h occurring in the middle of the day. Replicating ambient UV-B levels has been shown to be lethal for the tadpoles of some species (van Uitregt et al., 2007), and so UV-B levels were ~6% of ambient radiation to enable us to investigate non-lethal effects (Alton et al., 2012; Alton et al., 2010) while maintaining sufficient survival rates (Alton et al., 2011; van Uitregt et al., 2007). Tadpoles in the UV– treatment were exposed to the same lighting regime generated by generic linear fluorescent bulbs (UV-B 8.5±1.5 µW cm^{-2}). The cumulative total radiation (UV-B) received over 24 h was 7817 and 3950 J m^{-2} for UV+ and UV– treatments, respectively. In summary, tadpoles were exposed to one of four treatment groups: 24°C/UV+, 24°C/UV–, 18–32°C/UV+ and 18–32°C/UV–.

We recorded survival to stage 35–37 (Gosner, 1960) and the time taken to reach this stage. Stage 35–37 is a relatively stable time in development (Gosner, 1960) before hindlimbs are large enough to effect swimming movement (Hoff and Wassersug, 2000). At this developmental stage, tadpoles were tested for CT_{max}, or at one of five temperatures for RMR or maximum burst swimming performance. We then recorded body mass (g) and body and

Table 3. ANOVA table from non-linear regression on maximum burst swimming data

	a			*b*			*c*		
	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value
Temperature	1,150	3.69	0.06	1,150	0.03	0.86	1,150	2.58	0.11
UV	1,150	0.58	0.45	1,150	0.82	0.37	1,150	0.57	0.45
Tail length	1,150	6.44	0.01	1,150	0.49	0.49	1,150	0.004	0.95
Temperature × UV interaction	1,150	2.78	0.10	1,150	0.003	0.95	1,150	0.45	0.50

Data are for parameters from the function: $y=ax^2+bx+c$.

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Table 4. Q_{10} values for maximum burst swimming performance for *P. ornatum* tadpoles in thermally stable or thermally variable treatments, with or without UV-B radiation

	18–32°C/UV–	18–32°C/UV+	24°C/UV–	24°C/UV+
Q_{10} (18–38°C)	1.17	1.06	1.07	1.08
Q_{10} (18–28°C)	1.40	1.30	0.98	0.88
Q_{10} (28–38°C)	0.98	0.86	1.18	1.32

tail length (mm). We randomly selected one tadpole from each replicate container to be assessed for each performance measure so that each replicate had a tadpole included in each test at each temperature. After testing, tadpoles were killed by exposure to Aquis-S (Aquis-S New Zealand Ltd).

CT_{max}

CT_{max}, the temperature at which animals lose the ability to escape from conditions that may ultimately lead to death, was determined using the dynamic method (Lutterschmidt and Hutchinson, 1997; Duarte et al., 2012). Briefly, tadpoles were exposed to a constant heating rate of 0.5°C min^{-1} in a waterbath (24–43.7°C) until they no longer responded to mechanical stimulation with blunt forceps. At this time they were immediately transferred to water at room temperature to allow recovery. CT_{max} measurements were non-fatal and all tadpoles recovered.

RMR

RMR was calculated from oxygen consumption using closed system respirometry (Sinclair et al., 2006) at five test temperatures (18, 24, 28, 33 and 38°C) to generate a thermal dependence curve. To prevent thermal shock, tadpoles were brought to the test temperature at a rate of 4°C h^{-1} and allowed to adjust to the test temperature for 1 h. Tadpoles were then placed individually into 25 ml plastic respirometers (syringes) filled with air-saturated, dechlorinated aged water. Respirometers were submerged in a waterbath set to the test temperature (±0.5°C), and after 10 min (to allow tadpoles to recover from handling), respirometers were sealed with three-way taps and left for 40–90 min, depending on the test temperature (higher temperatures require less time). The respirometers were fitted with an oxygen-sensitive fluorescent Sensor Spot (PreSens, Regensburg, Germany) and oxygen partial pressure was determined non-invasively by measuring the fluorescence of the sensor spot through the plastic wall of the respirometer. A fibre-optic cable connected to a Fibox3 reader was used to capture and record fluorescence readings. Continuous, simultaneous temperature recordings of the waterbath allowed for the correction of O_2 solubilities with changing water temperature.

Oxygen consumption rate ($\dot{V}_{O_2}$: ml O_2 h^{-1}) was calculated using the following formula:

$$\dot{V}_{O_2} = (\Delta O_2 \times V) / T, \quad (1)$$

where ΔO_2 is the change in oxygen in the chamber (ml O_2 l^{-1}), V is the volume of the respirometer container (l) and T is time (min).

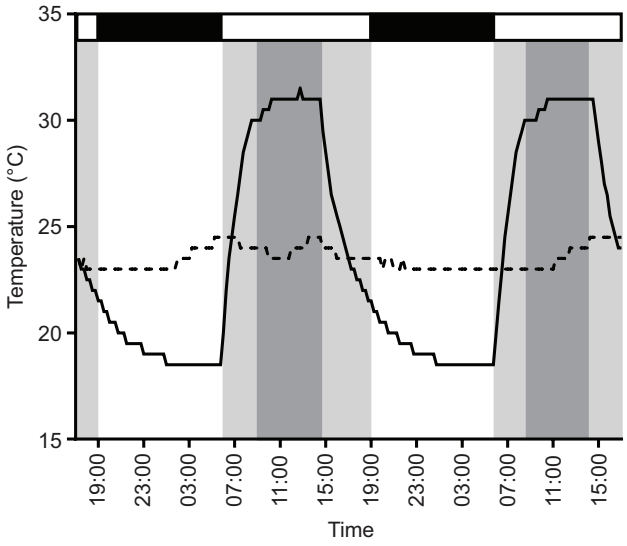

Fig. 6. Temperature and UV-B treatments. *Platyplectrum ornatum* tadpoles developed in either fluctuating (18–32°C; solid line) or constant (24°C; dashed line) temperature treatments. Tadpoles in UV+ treatments were exposed to UV-B radiation for 13 h per day (light grey; 06:00 h–19:00 h) with an additional high UV-B peak for 5 h per day (dark grey; 36±4 μW cm^{-2} between 10:00 h and 15:00 h). Tadpoles in UV treatments were exposed to this same lighting regime using generic fluorescent bulbs (8.5±1.5 μW cm^{-2} between 06:00 h and 19:00 h). The black and white bar represents photoperiod.

Burst swimming performance

Burst swimming performance was assessed at five temperatures (18, 24, 28, 33 and 38°C) to generate a thermal dependence curve. As for RMR, tadpoles were brought to each test temperature slowly to prevent thermal shock. Burst swimming performance was assessed in a swimming arena (27×13×5 cm) lined with reflective tape to give a clear silhouette of each tadpole. This container was filled with dechlorinated aged tapwater to 3 cm depth to prevent vertical movement, and semi-submerged in a waterbath set to the test temperature. Startle responses (C-start responses) were elicited by touching the tadpole's head with a blunt probe and recorded using a high-speed digital camera (Canon EX-FH25) recording at 240 Hz, pointed at a mirror positioned at a 45 deg angle above the burst arena. The first 100 ms following the completion of the C-start were analysed (Tracker Video Analysis and Modelling Tool, Open Source Physics) frame-by-frame by digitising the snout to determine maximum velocity. Three startle responses were recorded for each tadpole and individual burst swimming data were smoothed using a generalised cross-validatory quantic spline filter (Walker, 1998). The fastest burst was recorded as maximum burst performance (U_{max}).

Statistical analysis

The thermal sensitivity of activity rates for RMR and burst swimming performance were calculated as $Q_{10}=(R_2/R_1)^{(10/T_2-T_1)}$, where R represents the rate at temperature (T) 1 and 2. Thermal sensitivities were calculated for the entire range of test temperatures, as well as for the upper (28–38°C) and lower (18–28°C) temperature ranges.

Morphometric, survival and CT_{max} data were analysed using generalised linear models including temperature, UV-B treatment and an interaction term in the maximal model. For RMR and burst swimming performance, non-linear regression was used to fit data to a quadratic function to describe the thermal performance curves. Test temperature, temperature treatment, UV-B treatment and an interaction term were included in the maximal modal as well as body mass and body length, respectively, as co-variates. All analyses were done using the R statistical software package (R Development Core Team, 2007). Where indicated, Bonferroni's *post hoc* pairwise analyses were used. Data are presented as means ± s.e.m.

Competing interests

The authors declare no competing financial interests.

Author contributions

P.K and C.E.F designed the experiment; C.E.F provided materials; P.K performed the experiments, analysed the data and wrote the manuscript; C.E.F and R.L.C edited the manuscript.

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