© 2015. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2015) 218, 1402-1409 doi:10.1242/jeb.116558

RESEARCH ARTICLE

Developmental thermal plasticity of prey modifies the impact of predation

Frank Seebacher* and Veronica S. Grigaltchik

ABSTRACT

Environmental conditions during embryonic development can influence the mean expression of phenotypes as well as phenotypic responses to environmental change later in life. The resulting phenotypes may be better matched to their environment and more resilient to environmental change, including human-induced climate change. However, whether plasticity does improve success in an ecological context is unresolved. In a microcosm experiment, we show that developmental plasticity in embryos of the frog *Limnodynastes peronii* is beneficial by increasing survivorship of tadpoles in the presence of predators when egg incubation (15 or 25°C) and tadpole acclimation temperature in microcosms (15 or 25°C) coincided at 15°C. Tadpoles that survived predation were smaller, and had faster burst swimming speeds than those kept in no-predator control conditions, but only at high (25°C) egg incubation or subsequent microcosm temperatures. Metabolic rates were determined by a three-way interaction between incubation and microcosm temperatures and predation; maximal glycolytic and mitochondrial metabolic capacities (enzyme activities) were lower in survivors from predation compared with controls, particularly when eggs were incubated at 25°C. We show that thermal conditions experienced during early development are ecologically relevant by modulating survivorship from predation. Importantly, developmental thermal plasticity also impacts population phenotypes indirectly by modifying species interactions and the selection pressure imposed by predation.

KEY WORDS: Acclimation, Climate change, Fish, Locomotor performance, Species interactions, Survivorship, Tadpoles

INTRODUCTION

Environmental conditions during embryonic development can influence the mean expression of phenotypes (Levins, 1968; Bateson, 2001; Burton and Metcalfe, 2014; Mateus et al., 2014). The resulting phenotypic plasticity is usually non-reversible within organisms, and it is advantageous if the embryonic environment is a good predictor of conditions experienced later in life (Levins, 1968; Bateson et al., 2014; Nettle et al., 2013; Pigliucci, 2005). Hence, if environmental cues experienced early in life predict environmental conditions experienced at later life history stages, offspring should have increased fitness in that environment (Levins, 1968). However, there are limits and costs to developmental plasticity that may constrain its evolution (Auld et al., 2010; DeWitt et al., 1998; Pigliucci, 2005; Relyea, 2002). The cost of plasticity – that is, fitness decrements resulting from plasticity – is thought to include the energetic investment necessary to maintain the regulatory mechanisms necessary to enable the expression of plastic traits (DeWitt et al., 1998). Limits to plasticity – that is, failure to express optimal phenotypes – may manifest as a mismatch between the expressed phenotype and environmental conditions. The latter depends on the predictability of the environment, and greater variation between pre-zygotic and later offspring environments will increase the likelihood of a phenotype–environment mismatch (Botero et al., 2015; DeWitt et al., 1998). However, in addition to developmental plasticity, which is more or less non-reversible during later life history stages, adult phenotypes may change reversibly in response to environmental fluctuation. Interestingly, recent empirical evidence suggests that developmental and reversible plasticity (acclimation) are linked (Scott and Johnston, 2012; Seebacher et al., 2014). If this were the case, it would be expected that the correspondence between the embryonic and later environments becomes less important, and that individuals can respond to short-term variation in addition to, or in spite of, imprinting during early development (Gabriel, 2005; Lande, 2014; Seebacher et al., 2014).

The resulting phenotypic plasticity in physiological traits such as locomotor performance or metabolism may render individuals more resilient to environmental change, including human-induced climate change (Schulte, 2013). For example, maintaining high levels of locomotor performance across environmental gradients could improve escape from predators (Irschick and Losos, 1998). The next challenge in the field lies in determining how plasticity affects interactions between species (Schmitz and Barton, 2014).

Changing environments can change trophic interactions, which depend on the physiology that underlies reproduction, growth rates and movement of prey, and the energy requirements and abundance of predators (Post, 2013; Traill et al., 2010; Wilmers et al., 2007). Physiological responses of individuals to environmental change may therefore alter species interactions if the interacting species respond asynchronously to the change (Durant et al., 2007; Edwards and Richardson, 2004; Grigaltchik et al., 2012). The relationship between plasticity induced during embryonic development and acclimation during subsequent life history stages therefore may shape predator–prey interactions in variable environments. Our aim was to determine whether plasticity induced by the thermal environment experienced during embryonic development and subsequent life history stages modifies the impact of predation on tadpoles of the frog *Limnodynastes peronii* Duméril and Bibron 1841.

Predation is a major selection pressure that influences survivorship of prey, as well as the phenotypes of survivors if predators show preferences for particular prey characteristics. Predator presence may also induce plasticity in prey, and tadpoles in particular change their morphology and behaviour in response to predators (Pease and Wayne, 2014; Relyea, 2001; Van Buskirk and McCollum, 2000). At least some of the phenotypic characteristics of prey that alter the impact

School of Biological Sciences A08, University of Sydney, Sydney, NSW 2006, Australia.

*Author for correspondence (frank.seebacher@sydney.edu.au)

Received 10 November 2014; Accepted 5 March 2015

The Journal of Experimental Biology

The Journal of Experimental Biology (2015) 218, 1402-1409 doi:10.1242/jeb.116558

of predation are also dependent on temperature so that there may be an interaction between environmentally induced plasticity and predation (Abrahams et al., 2007; Traill et al., 2010). For example, voluntary activity may decrease both in the presence of predators (Pease and Wayne, 2014) and with decreasing temperature. However, burst swimming performance also decreases with decreasing temperature (Temple and Johnston, 1997), which may decrease success in predator escape (Domenici and Blake, 1997; Irschick and Losos, 1998; Watkins, 1996). Hence, plastic responses that compensate for the thermodynamic effect of decreasing temperature on physiological functions may be linked to survivorship by improving escape from predators. Metabolic traits parallel locomotor responses and provide fast glycolytic energy (ATP) release to facilitate burst performance, or slower sustained mitochondrial ATP production to support longer-lasting movement and growth. There may be a positive relationship between growth rate and temperature (Seebacher and Grigaltchik, 2014). However, often predators have a preference for larger individuals, and tadpoles of *Rana sylvatica* are smaller in the presence of predators (Van Buskirk, 1998). Predation can therefore act as a directional selection pressure that produces particular prey phenotypes, such as smaller size (Van Buskirk, 1998), lower metabolic rates (Handelsman et al., 2013; Krams et al., 2013) and greater locomotor performance (Watkins, 1996). All of these traits that respond to predation pressure are also sensitive to changes in temperature (Traill et al., 2010). Hence, it would be expected that plastic responses to thermal variability will also modulate the effects of predation on surviving prey phenotypes.

To test this prediction, we incubated freshly collected eggs from the frog *L. peronii* at different temperatures reflecting conditions near the seasonal extremes at the frogs' collection site. We then exposed the tadpoles in microcosms at the different incubation temperatures with and without predators in a fully factorial design. We tested the hypothesis that developmental modification of phenotypes is beneficial so that tadpoles have greater survivorship when incubation and subsequent microcosm temperatures coincide. Additionally, because predators may select particular phenotypes, we tested the hypothesis that survivors from predation will have greater locomotor performance and greater maximal metabolic capacities (enzyme activities), and lower metabolic rates indicative of lower levels of activity, and will be smaller than in the absence of predators. However, we also predicted that there will be an interaction between incubation and microcosm temperatures, and the effect of predation on survivor phenotypes, because animals for which incubation and microcosm temperatures coincide will be better matched to their environment and therefore suffer lower rates of predation so that predator-linked selection is weaker. We chose the striped marsh frog *L. peronii* as the study organism because tadpoles of *L. peronii* are known to acclimate swimming performance and metabolism (Rogers et al., 2004; Wilson and Franklin, 1999). Also, the species occurs across a broad latitudinal range along the east coast of Australia (Schäuble, 2004; Wilson, 2001), and breeds for most of the year (Schell and Burgin, 2003; Tyler, 2011). The species lives in shallow ponds and eggs are laid and hatch in foam nests at the water surface. Embryonic development of *Limnodynastes* in the egg is fairly rapid (3–5 days) but the tadpole stage can be prolonged (Lane and Mahony, 2002). Tadpoles hatched late in summer are likely to overwinter and metamorphose in the next spring.

RESULTS

Survivorship

The three-way interaction (incubation×microcosm×predator interaction; Table 1, Fig. 1) shows that in the absence of predators, survivorship did not differ between incubation temperatures in either cold or warm microcosms (both *post hoc* $P>0.95$). However, survivorship was greater in cold microcosms compared with warm microcosms in tadpoles from both cold and warm incubation temperatures (both *post hoc* $P<0.005$). Survivorship decreased in the presence of predators in both cold (*post hoc* $P<0.01$) and warm microcosms (*post hoc* $P<0.0001$) compared with no-predator control tanks. Cold microcosms had greater survivorship than warm microcosms (*post hoc* $P<0.0001$), and incubation temperature did not affect survivorship in warm microcosms (*post hoc* $P=0.13$). Interestingly, however, survivorship in cold microcosms was significantly greater in tadpoles that were incubated at cold temperatures compared with those incubated at warm temperatures (*post hoc* $P<0.005$).

Table 1. Results of permutational ANOVA testing for the effects of developmental (incubation) temperature, acclimation temperature in different microcosms and predator presence on tadpole survivorship and total length

	Survivorship		Total length	
Source	$SS_{1,42}$	*P*	$SS_{1,127}$	*P*
Incubation	0.13	0.003	4.49	0.26
Microcosm	3.35	<0.0001	43.15	<0.0001
Predator	2.20	<0.0001	217.79	<0.0001
Incubation×microcosm	0.16	0.0008	5.65	0.33
Incubation×predator	0.14	0.0008		
Microcosm×predator	0.0068	0.39	**38.88**	**0.002**
Incubation×microcosm×predator	**0.19**	**<0.0001**		

Minimal models determined by the Akaike information criterion (AIC) are shown for each response variable, as well as sums of squares with degrees of freedom as subscripts, and permutational probabilities. The relevant terms for interpretation of data are shown in bold.

Size

Total length of surviving tadpoles differed significantly between microcosm temperatures (microcosm×predator interaction; Table 1,

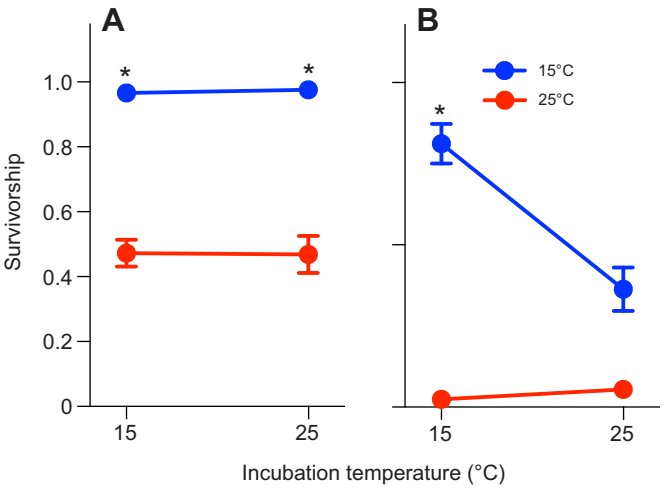

Fig. 1. Survivorship of tadpoles developed from eggs incubated at 15 or at 25°C and then raised in microcosm tanks at 15 or 25°C. There was a significant three-way interaction between incubation temperature, microcosm temperature and predation by mosquitofish (*Gambusia holbrooki*). (A) In the absence of predators, tadpoles in cold (15°C) microcosms had significantly greater survivorship compared with those in warm (25°C) microcosms (significant differences are indicated by asterisks). (B) The presence of predators decreased survivorship, but survivorship of tadpoles from 15°C incubation treatments in 15°C microcosms was significantly greater than that of tadpoles from other treatments. Means±s.e. are shown and sample sizes are 5–8 microcosms per treatment group.

The Journal of Experimental Biology (2015) 218, 1402-1409 doi:10.1242/jeb.116558

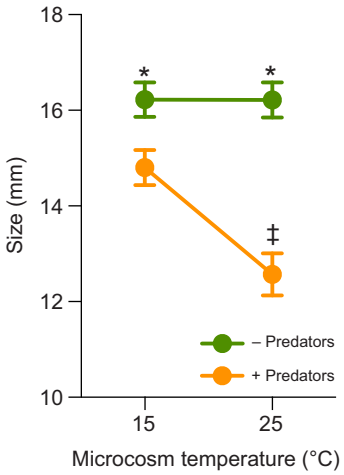

Fig. 2. Total body length of tadpoles exposed to predation by *G. holbrooki*. There was a two-way interaction between microcosm temperature and predation; survivors from predation were significantly smaller than no-predator controls (indicated by asterisks), and in the presence of predators tadpoles were significantly smaller in 25°C compared with 15°C microcosms (indicated by double dagger). Means±s.e. are shown and sample sizes are 13–19 tadpoles for each experimental group.

Fig. 2). Tadpoles were significantly smaller in the presence of predators compared with no-predator controls (*post hoc* $P<0.005$). In the presence of predators, tadpoles were significantly smaller in 25°C compared with 15°C microcosms (*post hoc* $P<0.0001$), but there was no difference between microcosms in the absence of predators (*post hoc* $P=0.96$).

Swimming performance

Both swimming speed and acceleration increased with increasing body length (main effect of body length, Table 2). The significant interaction between incubation temperature and predator presence (Table 2, Fig. 3A) shows swimming speeds of tadpoles from warm incubation temperatures that survived predators were significantly higher compared with no-predator controls ($P<0.0001$); predation did not affect swimming performance in cold-incubated tadpoles (*post hoc* $P=0.27$; Fig. 3A). Similarly, tadpoles surviving predation in warm microcosms had significantly greater swimming speeds compared with controls (microcosm×predation interaction; Table 2, Fig. 3B; *post hoc* $P<0.0001$), but there were no differences in cold microcosms (*post hoc* $P=0.27$).

Tadpoles from cold incubation temperatures reared in warm microcosms had the highest swimming performance (incubation×microcosm interaction, Table 2, Fig. 3C; *post hoc* $P<0.0001$), but there were no differences between microcosm conditions in swimming performance of tadpoles from warm incubation conditions (*post hoc* $P=0.075$). Tadpoles from different microcosm temperatures performed differently at different test temperatures (microcosm×test temperature interaction; Table 2, Fig. 3D). Tadpoles from warm microcosms swam faster at both test temperatures compared with those from cold microcosms (*post hoc* 15°C test temperature, $P<0.01$; 25°C test temperature, $P<0.0001$), and swimming performance increased with increasing test temperature in tadpoles from warm microcosms (*post hoc* $P<0.0001$), but not in those from cold microcosms (*post hoc* $P=0.93$; Fig. 3D).

Similar to swimming speed, acceleration of tadpoles from warm incubation temperatures that survived predators was significantly higher compared with no-predator controls (incubation×predation interaction; Table 2, Fig. 3E; *post hoc* $P<0.0001$), but predation did not affect acceleration in cold-incubated tadpoles (*post hoc* $P=0.79$; Fig. 3E). Additionally, acceleration was significantly higher in tadpoles from warm microcosms compared with cold microcosms (microcosms main effect; Table 2; cold: 106.83±2.35 BL s^{-2}, warm: 127.52±3.36 BL s^{-2}, means±s.e.).

Metabolic rate

Metabolic rate increased with increasing test temperature (main effect of test temperature; Table 2; cold: 0.15±0.0080 µmol g^{-1} min^{-1}, warm: 0.23±0.010 µmol g^{-1} min^{-1}). However, metabolic rate differed significantly with incubation and microcosm temperatures, and the effect of predation (incubation× microcosm×predation interaction; Table 2, Fig. 4). In the absence of predators, metabolic rates were higher in cold-incubated tadpoles from cold microcosms compared with warm microcosms (*post hoc* $P<0.005$), but there were no differences between microcosms in tadpoles incubated at warm temperatures (*post hoc* $P=0.80$; Fig. 4A). The pattern was reversed in survivors from predation, and there were no differences between cold-incubated tadpoles (*post hoc* $P=0.63$), but warm-incubated tadpoles had significantly higher metabolic rates in cold microcosms (*post hoc* $P<0.03$; Fig. 4B).

Table 2. Results of permutational ANOVA testing for the effects of developmental temperature, microcosm temperature, predator presence and acute test temperature (T_{test}) on tadpole swimming performance and routine metabolic rates

	Swimming speed		Acceleration		Metabolic rate	
Source	$SS_{1,248}$	*P*	$SS_{1,248}$	*P*	$SS_{1,262}$	*P*
Total length	**0.089**	**<0.0001**	**1.80**	**<0.0001**		
Incubation	0.00078	0.35	0.50	0.02	0.017	0.26
Microcosm	0.043	<0.0001	**2.37**	**<0.0001**	0.063	<0.0001
T_{test}	0.018	<0.0001	0.35	0.074	**0.41**	**<0.0001**
Predator	0.00003	0.97	0.003	0.86	0.011	0.38
Incubation×microcosm	**0.016**	**0.007**	0.001	0.96	0.0044	0.92
Incubation×T_{test}	0.0021	0.15	0.072	0.17	0.0090	0.82
Incubation×predator	**0.018**	**<0.0001**	**2.09**	**<0.0001**	0.0041	0.91
Microcosm×T_{test}	**0.011**	**0.004**			0.00034	0.98
Microcosm×predator	**0.0071**	**0.03**			0.0018	0.92
Predator × T_{test}					0.014	0.11
Incubation×microcosm×predator					**0.042**	**0.009**

Minimal models determined by the AIC are shown for each response variable, as well as sums of squares with degrees of freedom as subscripts and permutational probabilities. The relevant terms for interpretation of data are shown in bold.

The Journal of Experimental Biology (2015) 218, 1402-1409 doi:10.1242/jeb.116558

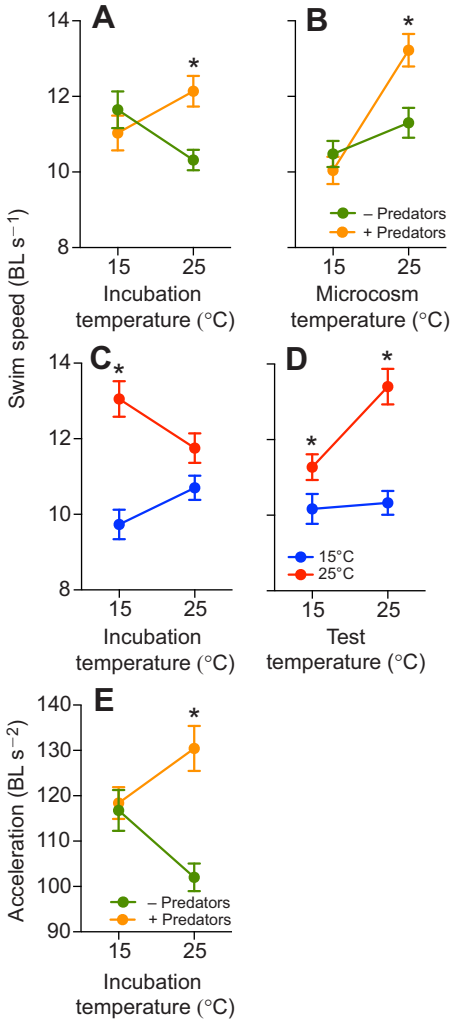

Fig. 3. Burst swimming performance and acceleration of tadpoles developed from eggs incubated at 15 or 25°C, and then kept in microcosm tanks at 15 or 25°C. (A–D) Burst swimming performance; (E) acceleration. Exposure to *G. holbrooki* predators altered responses to incubation and microcosm temperature (two-way interactions; A,B). Surviving tadpoles that were incubated at 25°C were significantly faster than no-predator controls (A; significant differences are indicated by asterisks). Similarly, survivors were faster than no-predator controls in 25°C microcosms (B). Swimming speed of tadpoles incubated at 15°C was significantly faster in warm microcosms (25°C, red symbols and line) than in cold microcosms (15°C, blue symbols and line; two-way interaction, C), and tadpoles from warm microcosms were overall faster than those from cold microcosms (D). Acceleration of tadpoles showed a similar two-way interaction between incubation temperature and predation to swimming speed (E). Means±s.e. are shown and sample sizes are 12–18 tadpoles for each experimental group.

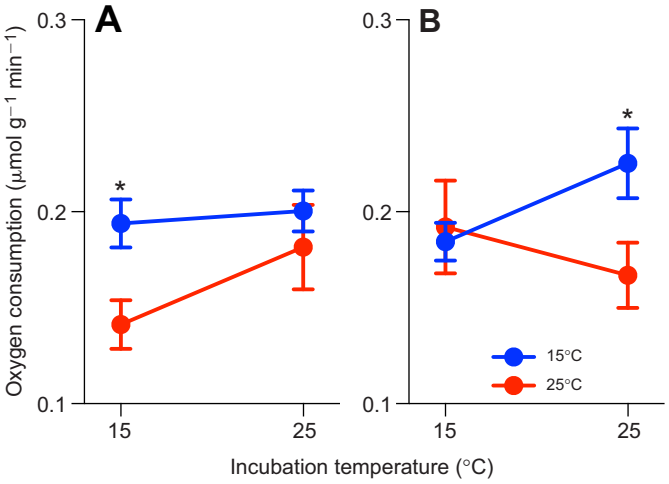

Fig. 4. Metabolic rates of tadpoles developed from eggs incubated at 15 or 25°C, and then kept in microcosm tanks at 15 or 25°C. There was a three-way interaction between incubation and microcosm temperatures and exposure to predators. In the absence of predators, cold-incubated tadpoles were faster in cold microcosms (A, indicated by an asterisk), but the pattern was reversed in the presence of predators (B). Means±s.e. are shown and sample sizes are 14–22 tadpoles for each experimental group.

Enzyme activities

Activities of the glycolytic enzyme lactate dehydrogenase and the mitochondrial enzymes citrate synthase and cytochrome *c* oxidase increased with test temperature (test temperature main effects; Table 3, Fig. 5A–C). Citrate synthase activity was greater in tadpoles from warm microcosms, but the reverse was the case for cytochrome *c* oxidase (microcosm main effects; Table 3, Fig. 5B,C).

Both lactate dehydrogenase and citrate synthase activities were lower in tadpoles from 25°C incubation treatments that survived predation compared with no-predator controls (incubation×predator interactions; Table 3, Fig. 5D,E; both *post hoc* $P<0.02$); there was no difference in activities in tadpoles that were incubated at 15°C (Fig. 5D,E; both *post hoc* $P>0.6$). Cytochrome *c* oxidase activity was slightly but significantly higher in tadpoles that survived predation (2.47±0.12 μmol g^{-1} min^{-1}) compared with no-predator controls (2.18±0.10 μmol g^{-1} min^{-1}; predator main effect; Table 3).

DISCUSSION

We have shown that the thermal environment experienced by developing organisms can affect interspecific interactions. Developmental plasticity is beneficial at least in cool conditions where survivorship is increased when the environment of offspring coincides with the embryonic environment. Tadpoles hatched late in the season overwinter and metamorphose in the next spring (Lane and Mahony, 2002; Schell and Burgin, 2003). Hence, tadpoles hatching late in the season would experience cool egg temperatures and also cool temperatures throughout most of their tadpole phase. Increased survivorship under cool conditions is therefore advantageous for overwintering animals. However, tadpoles that hatch early in the season in spring will develop in warming environments before metamorphosing. Maximising performance at the same environment as that experienced by embryos therefore would not be advantageous in the latter case. However, survivorship was much lower in the warm microcosms, both in absolute numbers and relative to control. This could imply a negative effect of the warm environment per se, which is unlikely given the broad latitudinal distribution of the species and its shallow pond habitat (Wilson, 2001). It is more likely that predation pressure was greater under warm conditions, which could be explained at least partly by the greater motivation to feed of predators in warmer environments regardless of their thermal history (Grigaltchik et al., 2012). Hence, it is possible that greater predation pressure masked the effect of plasticity on survivorship in the warm microcosms. Rather than just representing an artefact of the experimental design, these results may reflect that the benefits of developmental plasticity are context dependent; in this case, benefits are apparent under low predation pressure but may disappear as predation pressure increases.

The Journal of Experimental Biology (2015) 218, 1402-1409 doi:10.1242/jeb.116558

Table 3. Results of permutational ANOVA testing for the effects of developmental temperature, microcosm temperature, predator presence and acute test temperature on tadpole metabolic enzyme activities

	LDH		CS		COX	
Source	$SS_{1,135}$	*P*	$SS_{1,135}$	*P*	$SS_{1,135}$	*P*
Incubation	13.21	0.1	2.57	0.009	0.0090	0.92
Microcosm	26.2	0.11	**12.19**	**<0.0001**	**4.94**	**<0.0001**
T_{test}	**4443.70**	**<0.0001**	**80.54**	**<0.0001**	**43.44**	**<0.0001**
Predator	113.45	<0.01	5.88	<0.0001	**3.10**	**0.04**
Incubation×microcosm	6.1	0.56	0.27	0.44	0.43	0.54
Incubation×predator	**189.00**	**<0.0001**	**4.88**	**0.008**	0.19	0.73

LDH, lactate dehydrogenase; CS, citrate synthase; COX, cytochrome *c* oxidase.
Minimal models determined by the AIC are shown for each response variable, as well as sums of squares with degrees of freedom as subscripts and permutational probabilities. The relevant terms for interpretation of data are shown in bold.

The change in environmental conditions between embryonic and later life history stages is also thought to render developmental plasticity relatively ineffective in compensating for climate change, because later environmental conditions are not predictable (Visser, 2008). However, embryonic conditions also modulate the capacity for reversible acclimation at later life history stages (incubation×microcosm interactions; Scott and Johnston, 2012; Seebacher et al., 2014). Hence, even if survivorship is low, compensation for environmental variability in physiological traits as a result of acclimation may increase reproductive output of the surviving animals and thereby confer a benefit for the population. This suggestion is speculative and would be interesting to test experimentally.

Even though growth is maximised at warm temperatures in *L. peronii* (Seebacher and Grigaltchik, 2014), tadpoles in the warm

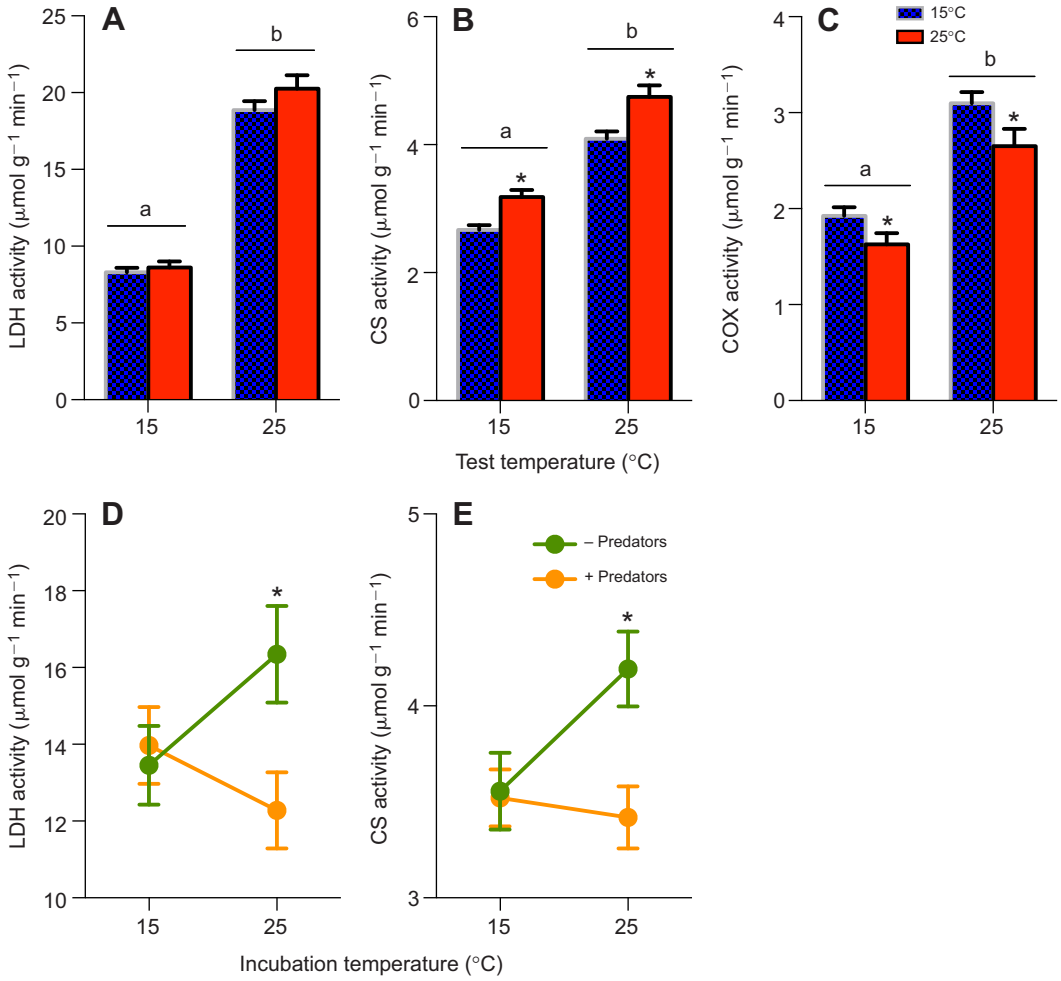

Fig. 5. Activities of the glycolytic enzyme lactate dehydrogenase (LDH), and mitochondrial enzymes citrate synthase (CS) and cytochrome *c* oxidase (COX). Activities were greater at 25°C acute test temperature compared with 15°C for all enzymes (A–C, indicated by different letters above horizontal bars). Tadpoles from warm microcosms (red bars) had higher CS activities than those from cold microcosms (blue bars; B), and this pattern was reversed for COX (C; significant differences are indicated by asterisks). There were two-way interactions between incubation temperature and predation for LDH and CS, and in both cases survivors from predation incubated at 25°C had lower activities compared with no-predator controls (D,E). Means±s.e. are shown and sample sizes are 9 tadpoles for each experimental group.

The Journal of Experimental Biology (2015) 218, 1402-1409 doi:10.1242/jeb.116558

microcosms were smaller in the presence of predators. Predators can affect size of prey in several ways. A decrease in density of tadpoles resulting from predation would be expected to result in an increase in size as a result of decreased competition (Werner and Anholt, 1996). Hence, density effects alone are unlikely to explain our results. In contrast, predator presence may reduce feeding activity and thereby slow growth rates of tadpoles (Van Buskirk and Yurewicz, 1998). This explanation would be likely if tadpoles had greater energy requirements at higher temperatures so that the effect of reduced feeding would be more pronounced compared with that in cooler environments. However, our metabolic data indicate that this was not the case. The most parsimonious explanation therefore is that predators preferred larger prey so that the survivors were smaller than animals from control treatments (Relyea, 2002; Van Buskirk, 1998), and this effect would be more pronounced in the warmer microcosms where predation pressure was greater.

Survivorship may be closely linked to physiological capacities, and locomotor performance in particular is often associated with predator escape (Domenici et al., 2007). In the absence of predators, both swimming speed and acceleration were greater in tadpoles incubated at 15°C, which indicates that cool incubation temperatures enhance physiological mechanisms underlying swimming performance. Metabolic capacities (lactate dehydrogenase and citrate synthase activities) in our tadpoles, however, were greater at the higher incubation temperature, and their activities are therefore unlikely to explain differences in swimming performance. Intrinsic muscle function and calcium cycling are more likely candidates underlying increased locomotor performance (Berchtold et al., 2000; Seebacher and Walter, 2012), particularly because expression of fast and slow fibre types is sensitive to developmental conditions (von Hofsten et al., 2008). In the presence of predators, swimming speed and acceleration were greater in tadpoles from warm incubation conditions and in tadpoles from warm microcosms; again, enzyme activities showed the opposite pattern and are therefore unlikely to explain differences in swimming performance. It is tempting to conclude that predators selected slower animals so that survivor phenotypes had greater locomotor performance under these conditions (Watkins, 1996). Alternatively, predator cues during early development can change morphology, and in particular increase tail length and depth, which increases swimming speed and acceleration and thereby escape from predators (Van Buskirk and McCollum, 2000). Our experimental design could not distinguish between direct selection by predators and predator-induced morphological changes that increased the probability of escape from predators. Nonetheless, regardless of the mechanism, there is a consensus that faster tadpoles are less likely to be eaten, and we have shown that the developmental environment influences swimming speed and probability of escape. One aspect of our data that argues against predator-induced morphological changes as a mechanism in this context is that surviving tadpoles were smaller and faster, whereas predator-induced morphological changes tend to produce longer tails and overall larger size (Van Buskirk and McCollum, 2000).

Incubation temperature also modulated reversible plasticity of swimming performance, which means that the potential cost of developmental plasticity resulting from a mismatch between embryonic and later environments would be reduced. However, the direction of the developmental effect was the reverse of that expected; that is, cold-incubated tadpoles performed best in warm acclimation conditions, and warm-acclimated tadpoles performed better than cold-acclimated animals across test temperatures. It could be speculated that this pattern is advantageous for animals hatching from eggs laid early in the season in relatively cool water that develop as tadpoles at increasingly higher temperatures. However, the mechanisms underlying this pattern are not immediately obvious, other than that cold incubation conditions may produce proteins with greater thermal sensitivity.

Resting or routine metabolic rates represent energy expenditure on maintenance and growth and, in the case of routine metabolic rates, low levels of activity. It has been suggested that metabolic rates are linked positively to personality traits, such as boldness and activity, which would render individuals more susceptible to predation (Biro and Stamps, 2010; Handelsman et al., 2013). However, contrary to our predictions, survivors from predation did not have lower metabolic rates, but metabolic rates were determined by interactions between thermal regimes and the presence of predators, which are not easily explained.

We show that thermal conditions experienced during early development influence survivorship from predation. Importantly, developmental thermal plasticity also impacts population phenotypes indirectly by modifying species interactions and the selection pressure imposed by predation. These findings are important for understanding how animals respond to changing environments including human-induced climate change. Determining environmental effects on single species is important because it reveals the physiological capacities and environmental tolerances of that species. However, the ecological consequences of species' tolerances can be revealed only by elucidating interactions within the community (Gilman et al., 2010; Post, 2013; Tylianakis et al., 2008). Our data show that thermal plasticity is a significant factor in shaping interactions.

MATERIALS AND METHODS

Ethics statement

Animals were collected under NSW National Parks and Wildlife Service Scientific Licence SL100518. All experiments were conducted under approval from the University of Sydney Animal Ethics Committee (approval L04/5-2011/2/5530).

Study animals and microcosms

Freshly laid striped marsh frog (*L. peronii*) egg masses (N=8) were collected from ponds (19–21°C) around Sydney, NSW, Australia (151.2° E, 33.8°S) in the early morning. Egg masses typically contain hundreds of eggs. Each egg mass was divided evenly into two incubation treatments, a 'warm' temperature treatment (water temperature: 25.0±0.5°C; eight containers 30×20×20 mm) and a 'cold' temperature treatment (water temperature: 15.0±0.5°C; eight containers). These temperatures were selected as they fall within the range of thermal conditions naturally experienced by both *L. peronii* tadpoles (Wilson, 2001) and mosquitofish (Seebacher et al., 2014), which we used as a predator (see below). Egg masses were maintained in aerated and filtered water from their collection site (50%) mixed with aged tap water (i.e. tap water that was aerated for 24–48 h before use to eliminate chlorine). When tadpoles reached Gosner stage 20 (fully hatched, 2–5 days), individuals were selected from each incubation treatment and added to a microcosm at either the same temperature as for incubation or crossed to the other incubation temperature (N=100 tadpoles/microcosm; N=11 microcosms for the warm incubation×cold microcosm treatment, N=12 microcosms for the cold incubation×cold microcosm treatment, N=12 microcosms for the warm incubation×warm microcosm treatment, N=15 microcosms for the cold incubation×warm microcosm treatment); we ensured that tadpoles from each egg mass were distributed across all treatments to avoid clutch effects.

Microcosms were plastic tanks (35×60×27 cm) filled with aged, dechlorinated water and containing 1 cm of washed sand (Richgro Garden Products, Jandakot, WA, Australia) on the bottom. Each microcosm also contained a sponge filter attached to a pump for continuous aeration during the experiment, eight floating plastic plants and 100 flat black river stones

The Journal of Experimental Biology (2015) 218, 1402-1409 doi:10.1242/jeb.116558

(approximately 4 cm diameter×1 cm thickness; Richgro Garden Products). The floating plants provided shelter for feeding tadpoles, and the river stones provided shelter for tadpoles resting on the bottom.

Microcosms were kept in a constant temperature room, and microcosm temperatures were reached by gradually changing the room temperature from 20 to 15°C (±0.5°C) over 2 days, and at the same time increasing the temperature in the warm microcosms with submersible heaters (200 W, Aqua One, Aqua Pacific, Southampton, UK) by 2.5°C per day. All microcosms were kept at a constant 12 h:12 h light:dark cycle throughout experiments. Frozen then thawed lettuce was added daily *ad libitum* as food for tadpoles. Uneaten lettuce was removed daily, and water was topped up when needed as a result of evaporation, but otherwise the microcosms were left undisturbed.

Mosquitofish (*Gambusia holbrooki*) were caught from the wild around Sydney, NSW, Australia (151.2°E, 33.8°S) using hand nets. Fish were housed in plastic tanks (35×60×27 cm, *N*=6 tanks, 25 animals/tank) with both males and females (1:1) to simulate natural conditions. Fish were kept at 20°C for the first 2 weeks of exposure to the lab environment, after which they were acclimated to one of the microcosm temperatures (water temperature: 15.0±0.5 or 25.0±0.5°C; *N*=3 tanks/acclimation treatment) for 4 weeks. Acclimation treatments were conducted as described for microcosms above, and fish were kept on a 12 h:12 h light:dark cycle.

At the end of mosquitofish acclimation treatments, tadpoles had inhabited microcosms for 2 weeks post-hatching. At this time, three adult length-matched (2.78±0.03 cm) male mosquitofish were added to six to seven microcosms of each thermal treatment combination (predator treatment); fish were introduced to microcosms that were at the same temperature as their acclimation temperature. Hence, around half of the microcosms for each incubation×microcosm temperature combination comprised the predator treatment while the other half represented a no-predator control. After the addition of the mosquitofish, microcosms were maintained for 2 weeks with minimal disturbance (see above). After 2 weeks, all mosquitofish were removed and each microcosm was cleaned out carefully by removing all stones and floating plants so that all surviving tadpoles could be collected, measured and counted. We then collected 1–4 tadpoles randomly from each microcosm to measure whole-animal performance of surviving tadpoles from the predator treatments, and of tadpoles from the no-predator control microcosms (to give a total of 14–22 tadpoles per treatment).

Swimming performance

Individuals were placed into a plastic tray (40×25×5 cm) filled with water to a depth of 2 cm and were allowed to equilibrate for 15 min. Burst swimming responses were initiated by startling individuals by lightly tapping their tail to elicit an escape response (Wilson and Franklin, 1999). The ensuing responses were filmed from above with a camera (Quickcam Pro, Logitech, China) filming at 30 frames s^{-1}. Three responses were measured for each individual. Responses were analysed (in Tracker software, Open Source Physics, USA: http://www.opensourcephysics.org) and the maximum velocity and acceleration during the three trials was recorded for each tadpole (Wilson and Franklin, 1999). Each tadpole was tested at 15 and 25°C with 24 h between trials.

Oxygen consumption

Tadpoles were gently dried, weighed and placed into a plastic respirometer (43 ml). The respirometers were equipped with inflow and outflow ports through which aged oxygenated water was supplied before measurements started, and which could be closed off without disturbance to the animals. Inside each respirometer we placed an oxygen sensor spot (PSt3, PreSens, Regensburg, Germany) that was monitored with a fibre-optic cable (PreSens) mounted on the outside of the respirometer and connected to an oxygen meter (FIBOX3, PreSens). After tadpoles were placed into the respirometers, the respirometers were left in a temperature-controlled room set to the appropriate test temperature for at least one hour, which is sufficiently long for recovery from handling stress (Kern et al., 2014). After this resting period, the respirometers were sealed and oxygen concentrations were determined every 15 min for a total of 60 min. We used the slope in the decline in oxygen concentrations over the last 45 min of measurements to determine rates of oxygen consumption in μmol min^{-1} g^{-1} wet mass; oxygen consumption rates stabilised over that time period. Oxygen consumption of each tadpole was measured at 15 and 25°C with 24 h between trials. We also measured oxygen consumption in respirometers not containing tadpoles as controls, and we subtracted oxygen consumption by the controls from the experimental values. In the event, oxygen consumption by controls was kept to a minimum by routinely cleaning equipment and regular water changes. Tadpoles were not completely stationary during trials so that the measurements represent routine rates of oxygen consumption that represent a mixture of oxygen consumed during low levels of voluntary activity, and maintenance and growth.

Metabolic enzyme activities

Tadpoles (*N*=9/treatment) were killed in a solution of buffered MS222 (0.25 g l^{-1}, pH 7.0; Sigma, Sydney, NSW, Australia) and then quickly measured (total body length), dried and frozen (−80°C) for later analysis. For enzyme assays, whole tadpoles were homogenised in 19 w/v extraction buffer (50 mmol l^{-1} imidazole/HCl, 2 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} EDTA, 0.1% Triton X-100 and 1 mmol l^{-1} glutathione) for citrate synthase (CS) and cytochrome *c* oxidase (COX) assays, and further diluted to 1 in 100 (tissue in buffer) for lactate dehydrogenase (LDH) assays. Citrate synthase and cytochrome *c* oxidase are mitochondrial enzymes that set maximal capacities for oxidative ATP production. Lactate dehydrogenase is a glycolytic enzyme that converts pyruvate to lactic acid, thereby producing ATP rapidly in the absence of oxygen. All assays were conducted at 15 and 25°C according to published protocols (Rogers et al., 2007).

Statistical analysis

We analysed all data with permutational ANOVA in the LmPerm package (Wheeler, 2014) in R (R Development Core Team, 2013). We used a permutation analysis because it tests hypotheses based on the data per se rather than on assumed population parameters, which is preferable when the sample sizes are small relative to the total population (Drummond and Vowler, 2012). We analysed survivorship with incubation temperature, microcosm temperature and predator treatment as factors, and individual microcosms were the level of replication. We used the same factors for the analysis of total length, but we used individual tadpoles as level of replication. Swimming performance, acceleration, metabolic rate and enzyme activities were analysed with incubation temperature, microcosm temperature, predator treatment and test temperature as factors, and individual tadpoles as level of replication. Body length was used as a covariate in the analyses of swimming speed and acceleration. We optimised the models by initially running the full model and then determining the terms to be retained in the analysis by calculating Akaike's information criterion (AIC); we dropped terms from the full model until AIC was minimised (Crawley, 2007) using the 'step' command in R (R, 2013). We report sums of squares for each source of variation as well as permutational *P*-values. We conducted *post hoc* tests for simple main effects following significant interactions by comparing marginal means with a permutational analysis (Quinn and Keough, 2004). We estimated the false discovery rate using the Benjamini–Hochberg procedure (Noble, 2009), and ascertained that it was below 5% for the whole study.

Competing interests

The authors declare no competing or financial interests.

Author contributions

F.S. designed the study, analysed the data and wrote the manuscript. V.S.G. conducted experiments and revised the manuscript.

Funding

This work was funded by an Australian Research Council Discovery Grant to F.S. V.G.S. was supported by an Australian International Postgraduate Research Scholarship.

References

Abrahams, M. V., Mangel, M. and Hedges, K. (2007). Predator-prey interactions and changing environments: who benefits? *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 2095-2104.

Auld, J. R., Agrawal, A. A. and Relyea, R. A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proc. R. Soc. B Biol. Sci.* **277**, 503-511.
Bateson, P. (2001). Fetal experience and good adult design. *Int. J. Epidemiol.* **30**, 928-934.
Bateson, P., Gluckman, P. and Hanson, M. (2014). The biology of developmental plasticity and the predictive adaptive response hypothesis. *J. Physiol.* **592**, 2357-2368.
Berchtold, M. W., Brinkmeier, H. and Müntener, M. (2000). Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. *Physiol. Rev.* **80**, 1215-1265.
Biro, P. A. and Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends Ecol. Evol.* **25**, 653-659.
Botero, C. A., Weissing, F. J., Wright, J. and Rubenstein, D. R. (2015). Evolutionary tipping points in the capacity to adapt to environmental change. *Proc. Natl. Acad. Sci. USA* **112**, 184-189.
Burton, T. and Metcalfe, N. B. (2014). Can environmental conditions experienced in early life influence future generations? *Proc. R. Soc. B Biol. Sci.* **281**, 20140311-20140311.
Crawley, M. J. (2007). *The R Book*. Chichester, UK: John Wiley & Sons Ltd.
DeWitt, T., Sih, A. and Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* **13**, 77-81.
Domenici, P. and Blake, R. (1997). The kinematics and performance of fish fast-start swimming. *J. Exp. Biol.* **200**, 1165-1178.
Domenici, P., Lefrancois, C. and Shingles, A. (2007). Hypoxia and the antipredator behaviours of fishes. *Philos. Trans. R. Soc. B Biol. Sci.* **362**, 2105-2121.
Drummond, G. B. and Vowler, S. L. (2012). Different tests for a difference: how do we do research? *J. Physiol.* **590**, 235-238.
Durant, J. M., Hjermann, D. Ø., Ottersen, G. and Stenseth, N. C. (2007). Climate and the match or mismatch between predator requirements and resource availability. *Clim. Res.* **33**, 271-283.
Edwards, M. and Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* **430**, 881-884.
Gabriel, W. (2005). How stress selects for reversible phenotypic plasticity. *J. Evol. Biol.* **18**, 873-883.
Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W. and Holt, R. D. (2010). A framework for community interactions under climate change. *Trends Ecol. Evol.* **25**, 325-331.
Grigaltchik, V. S., Ward, A. J. W. and Seebacher, F. (2012). Thermal acclimation of interactions: differential responses to temperature change alter predator-prey relationship. *Proc. R. Soc. B Biol. Sci.* **279**, 4058-4064.
Handelsman, C. A., Broder, E. D., Dalton, C. M., Ruell, E. W., Myrick, C. A., Reznick, D. N. and Ghalambor, C. K. (2013). Predator-induced phenotypic plasticity in metabolism and rate of growth: rapid adaptation to a novel environment. *Integr. Comp. Biol.* **53**, 975-988.
Irschick, D. J. and Losos, J. B. (1998). A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. *Evolution* **52**, 219-226.
Kern, P., Cramp, R. L. and Franklin, C. E. (2014). Temperature and UV-B-insensitive performance in tadpoles of the ornate burrowing frog: an ephemeral pond specialist. *J. Exp. Biol.* **217**, 1246-1252.
Krams, I., Kivleniece, I., Kuusik, A., Krama, T., Freeberg, T. M., Mänd, R., Vrublevska, J., Rantala, M. J. and Mänd, M. (2013). Predation selects for low resting metabolic rate and consistent individual differences in anti-predator behavior in a beetle. *Acta Ethol.* **16**, 163-172.
Lande, R. (2014). Evolution of phenotypic plasticity and environmental tolerance of a labile quantitative character in a fluctuating environment. *J. Evol. Biol.* **27**, 866-875.
Lane, S. J. and Mahony, M. J. (2002). Larval anurans with synchronous and asynchronous development periods: contrasting responses to water reduction and predator presence. *J. Anim. Ecol.* **71**, 780-792.
Levins, R. (1968). *Evolution in Changing Environments*. Princeton, NJ: Princeton University Press.
Mateus, A. R. A., Marques-Pita, M., Oostra, V., Lafuente, E., Brakefield, P. M., Zwaan, B. J. and Beldade, P. (2014). Adaptive developmental plasticity: compartmentalized responses to environmental cues and to corresponding internal signals provide phenotypic flexibility. *BMC Biol.* **12**, 97.
Nettle, D., Frankenhuis, W. E. and Rickard, I. J. (2013). The evolution of predictive adaptive responses in human life history. *Proc. R. Soc. B Biol. Sci.* **280**, 20131343.
Noble, W. S. (2009). How does multiple testing correction work? Nat. *Biotechnol.* **27**, 1135-1137.
Pease, K. M. and Wayne, R. K. (2014). Divergent responses of exposed and naive Pacific tree frog tadpoles to invasive predatory crayfish. *Oecologia* **174**, 241-252.
Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends Ecol. Evol.* **20**, 481-486.
Post, E. (2013). *Ecology of Climate Change*. Princeton, NJ, USA: Princeton University Press.
Quinn, G. R. and Keough, M. J. (2004). *Experimental Design and Data Analysis for Biologists*, pp. 252-253. Cambridge, UK: Cambridge University Press.
R Development Core Team. (2013). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
Relyea, R. A. (2001). Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* **82**, 523-540.
Relyea, R. (2002). Costs of phenotypic plasticity. *Am. Nat.* **159**, 272-282.
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. A Mol. Integr. Physiol.* **137**, 731-738.
Rogers, K. D., Thompson, M. B. and Seebacher, F. (2007). Beneficial acclimation: sex specific thermal acclimation of metabolic capacity in the striped marsh frog (*Limnodynastes peronii*). *J. Exp. Biol.* **210**, 2932-2938.
Schäuble, C. S. (2004). Variation in body size and sexual dimorphism across geographical and environmental space in the frogs *Limnodynastes tasmaniensis* and *L.* peronii. *Biol. J. Linn. Soc.* **82**, 39-56.
Schell, C. B. and Burgin, S. (2003). Swimming against the current: the brown striped marsh frog *Limnodynastes peronii* success story. *Aust. J. Zool.* **32**, 401-405.
Schmitz, O. J. and Barton, B. T. (2014). Climate change effects on behavioral and physiological ecology of predator–prey interactions: Implications for conservation biological control. *Biol. Control* **75**, 87-96.
Schulte, P. M. (2013). What is environmental stress? Insights from fish living in a variable environment. *J. Exp. Biol.* **217**, 23-34.
Scott, G. R. and Johnston, I. A. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proc. Natl. Acad. Sci. USA* **109**, 14247-14252.
Seebacher, F. and Grigaltchik, V. S. (2014). Embryonic developmental temperatures modulate thermal acclimation of performance curves in tadpoles of the frog *Limnodynastes peronii*. *PLoS ONE* **9**, e106492.
Seebacher, F. and Walter, I. (2012). Differences in locomotor performance between individuals: importance of parvalbumin, calcium handling and metabolism. *J. Exp. Biol.* **215**, 663-670.
Seebacher, F., Beaman, J. and Little, A. G. (2014). Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. *Funct. Ecol.* **28**, 137-148.
Temple, G. K. and Johnston, I. A. (1997). The thermal dependence of fast-start performance in fish. *J. Therm. Biol.* **22**, 391-401.
Traill, L. W., Lim, M. L. M., Sodhi, N. S. and Bradshaw, C. J. A. (2010). Mechanisms driving change: altered species interactions and ecosystem function through global warming. *J. Anim. Ecol.* **79**, 937-947.
Tyler, M. (2011). *Field Guide to the Frogs of Australia*. Canberra, Australia: CSIRO Publishing.
Tylianakis, J. M., Didham, R. K., Bascompte, J. and Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecol. Lett.* **11**, 1351-1363.
Van Buskirk, J. (1998). Selection for phenotypic plasticity in *Rana sylvatica* tadpoles. *Biol. J. Linn. Soc.* **65**, 301-328.
Van Buskirk, J. and McCollum, S. A. (2000). Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *J. Evol. Biol.* **13**, 336-347.
Van Buskirk, J. and Yurewicz, K. L. (1998). Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. *Oikos* **82**, 20-28.
Visser, M. E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proc. R. Soc. B Biol. Sci.* **275**, 649-659.
von Hofsten, J., Elworthy, S., Gilchrist, M. J., Smith, J. C., Wardle, F. C. and Ingham, P. W. (2008). Prdm1- and Sox6-mediated transcriptional repression specifies muscle fibre type in the zebrafish embryo. *EMBO Rep.* **9**, 683-689.
Watkins, T. B. (1996). Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. *Physiol. Zool.* **69**, 154-167.
Werner, E. E. and Anholt, B. R. (1996). Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. *Ecology* **77**, 157-169.
Wheeler, B. (2014). Package "lmPerm." R Package Version 1.1-2, 1-24.
Wilmers, C. C., Post, E. and Hastings, A. (2007). The anatomy of predator–prey dynamics in a changing climate. *J. Anim. Ecol.* **76**, 1037-1044.
Wilson, R. S. (2001). Geographic variation in thermal sensitivity of jumping performance in the frog *Limnodynastes peronii*. *J. Exp. Biol.* **204**, 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 Biochem. Syst. Environ. Physiol.* **169**, 445-451.