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Author/s:

Courtney Jones, SK;Munn, AJ;Penman, TD;Byrne, PG

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Long-term changes in food availability mediate the effects of temperature on growth, development and survival in striped marsh frog larvae: implications for captive breeding programmes

Stephanie K. Courtney Jones^{1,*}, Adam J. Munn¹, Trent D. Penman^{1,2} and Phillip G. Byrne¹

¹Institute for Conservation Biology and Environmental Management, School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia

²School of Ecosystem and Forest Science, University of Melbourne, Creswick, VIC 3363, Australia

*Corresponding author: Institute for Conservation Biology and Environmental Management, School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia. Tel: +61 2 4221 1932. Email: sk.courtneyjones@gmail.com

Food availability and temperature are known to trigger phenotypic change, but the interactive effects between these factors are only beginning to be considered. The aim of this study was to examine the independent and interactive effects of long-term stochastic food availability and water temperature on larval survivorship, growth and development of the striped marsh frog, *Limnodynastes peronii*. Larval *L. peronii* were reared in conditions of either constant or stochastic food availability and in water at three different temperatures (18, 22 and 26°C), and effects on survival, growth and development were quantified. Over the experimental period, larval growth rate was highest and survivorship lowest at the warmest temperature. However, changes in food availability mediated the effects of temperature, with slower larval growth and higher survivorship in stochastic food availability treatments. Tadpoles in the stochastic food availability treatments did not reach metamorphosis during the experimental period, suggesting that developmental stasis may have been induced by food restriction. Overall, these results demonstrate that changes in food availability alter the effects of water temperature on survival, growth and development. From an applied perspective, understanding how environmental factors interact to cause phenotypic change may assist with amphibian conservation by improving the number of tadpoles generated in captive breeding programmes.

Key words: Development, food availability, growth, metamorphosis, phenotypic plasticity, survival

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Introduction

Phenotypic plasticity is the ability of an organism to change its phenotype in response to varying abiotic and biotic environmental

factors (Miner *et al.*, 2005). When faced with dynamic environmental conditions, some organisms can readily respond by changing phenotypes, allowing for a range of optimal phenotypes to be produced in response to multiple environments

(DeWitt *et al.*, 1998). If the optimal phenotype can change with environmental conditions, this presents an adaptive advantage that can improve organismal fitness (De Jong, 2005; Reed *et al.*, 2010). Phenotypic plasticity has evolved in an array of organismal traits, but two traits that appear to be particularly plastic are growth (somatic growth) and development (ontogenic change; Relyea, 2001; Pfennig *et al.*, 2010). Plastic responses in growth and development can be triggered by various environmental factors. One environmental factor known to trigger plastic responses across a variety of taxa is food availability (see Monaghan, 2008; Munn *et al.*, 2010; Enriquez-Urzelai *et al.*, 2013; Rosen *et al.*, 2014). Empirical studies suggest that changes in food availability have long-term consequences for various life-history traits due to a reduction in the amount of energy that can be allocated to somatic growth (Yoneda and Wright, 2005; Inatsuchi *et al.*, 2010; Enriquez-Urzelai *et al.*, 2013).

Several theoretical models have considered how insufficient energy intake in conditions of reduced food availability might influence organismal growth and development and, ultimately, the probability of surviving and reproducing. The ‘metabolic down-regulation model’ predicts that food deprivation induces an overall metabolic depression that may occur as a physiological adaptation to reduce metabolic costs, via the down-regulation of metabolic rates, limiting processes such as growth and development (Keys *et al.*, 1950; Fischer and Linder Mayer, 2000; Rosen *et al.*, 2014). There is some empirical evidence to support this prediction. For example, in periods of stochastic food availability, coral reef fish exhibit a longer time to metamorphosis and smaller size at maturity (McLeod *et al.*, 2013; see also Nicieza and Metcalfe, 1997; Yoneda and Wright, 2005; Inatsuchi, Yamato and Yusa, 2010; Enriquez-Urzelai *et al.*, 2013). The ‘general optimization model’, a mathematical formalization of the ‘Wilbur–Collins model’, also predicts slower growth rate and longer developmental periods in response to poor growth conditions. However, this model proposes that developmental thresholds (such as minimum size) need to be attained prior to life-history transition (Day and Rowe, 2002). This model predicts that to meet minimum size thresholds for metamorphosis, individuals should extend the larval period (Day and Rowe, 2002).

The Wilbur–Collins model, developed explicitly for species that experience metamorphosis, also proposes that there should be a minimum threshold size at which developmental transitions occur (Wilbur and Collins, 1973; Day and Rowe, 2002). However, this model hypothesizes a trade-off between growth and development. The model predicts that in conditions of stochastic food availability, larval development is increased to evade the resource-poor environment, and growth rate is slowed, resulting in a smaller size at metamorphosis (Wilbur and Collins, 1973). This negative relationship between growth and metamorphosis has been reported in three spadefoot toad species (genus: *Scaphiopus*; Morey and Reznick, 2000). In low food availability conditions, larvae underwent earlier development to evade the resource-poor environment; however, a minimal threshold size had to be met before development could be expedited. Alternatively, if the

minimal threshold size was not met, larvae entered a developmental stasis (Morey and Reznick, 2000).

The effect of food availability on growth and development has also been explored using the stochastic dynamic programming (SDP) approach, which has been developed to determine an optimal strategy to maximize a particular fitness trait (Tenhumberg *et al.*, 2000). Using the SDP approach, Tenhumberg *et al.* (2000) developed an SDP model for a syrphid fly system to determine optimal size and age at maturity when exposed to stochastic food availability, but considered the timing of food availability during development, a novel inclusion largely ignored in other models of growth and development. In this SDP model, it is predicted that exposure to stochastic food availability throughout development would result in larvae pupating earlier and at a smaller size. Exposure to stochastic food availability in the early phase of development would result in syrphid larvae pupating later without altering size at pupation. In contrast, exposure to stochastic food availability conditions during the late phase of development would alter weight at pupation, not developmental time (Tenhumberg *et al.*, 2000), providing support for the notion that the timing of changes in food availability can control growth and development (see Nicieza and Metcalfe, 1997; Morey and Reznick, 2000, 2004; Yoneda and Wright, 2005; Inatsuchi *et al.*, 2010; Enriquez-Urzelai *et al.*, 2013).

Importantly, while the aforementioned models examine the effects of food availability on growth and development, multiple environmental factors can affect these life-history traits, and the effects of these factors are rarely independent. For instance, in temporal water bodies, plastic responses in growth and development may be triggered not only by food availability, but also by temperature (Leips and Travis, 1994; Sanuy *et al.*, 2008). The ‘temperature-size rule’ predicts that ectothermic species reared at cold temperatures display slow growth rates and a prolonged larval period, resulting in a larger size at metamorphosis (Kozłowski *et al.*, 2004). The temperature–size rule has been widely tested, and there is now considerable empirical evidence to suggest that this rule applies to the vast majority of ectothermic animals (Angilletta *et al.*, 2004; Walters and Hassall, 2006).

While the independent effects of food availability and temperature on growth and development in larval species are well established (Inatsuchi *et al.*, 2010), the interactive effects of these factors on growth, development and survival to maturity are only beginning to be considered (Álvarez and Nicieza, 2002a). One of the few models considering the interaction between environmental factors is the ‘fixed-rate model’ (Travis, 1984). The model postulates that while food availability may regulate specific life-history traits, such as larval growth and size at metamorphosis, the developmental rate becomes fixed at a certain point (Travis, 1984; Rose, 2005). However, the length of larval period can be regulated by other environmental factors, such as temperature (Álvarez and Nicieza, 2002b). There is some experimental evidence for interactive effects. For example, in a study investigating the

interactive effects of diet type and temperature on larvae of the Iberian painted frog (*Discoglossus galganoi*), it was found that larval period was extended with cooler temperatures; however, size at metamorphosis was regulated by the interaction between temperature and diet type (Álvarez and Nicieza, 2002b). More specifically, when exposed to plant- or animal-based diets, size at metamorphosis varied inversely to temperature, and although diet did not influence size at 12°C, exposure to the animal-based diet resulted in bigger metamorphs at 17 and 22°C (Álvarez and Nicieza, 2002b).

To date, few studies have investigated how food availability, and interactions between food availability and temperature, influence growth and development in ectotherms. Nevertheless, evidence is emerging to suggest that such interactions can alter developmental trajectories. Newman (1998) conducted a dietary experiment using spadefoot toad tadpoles (*Scaphiopus couchii*) and demonstrated that an abrupt change in food level during development had significant effects on an individual's age and size at metamorphosis. However, the magnitude and direction of these effects differed depending on the environmental temperature and tadpole density. More recently, in a study of coral reef fish McLeod *et al.* (2013) manipulated food availability by increasing time lags between feeding, at increasing temperatures. Overall, lower feeding regimens decreased survivorship to adulthood, and longer time to metamorphosis was observed. However, in their study McLeod *et al.* (2013) noted that predictable time lags between food supply may not be symptomatic of natural food supplies, indicating the importance of investigating the influence of stochastic food availability. Furthermore, changes in food availability occurring throughout the entire developmental period have received limited empirical attention (see Leips and Travis, 1994). Using an SDP model approach, Tenhumberg *et al.* (2000) considered the effects of timing of changes in food availability on the optimal size at maturity in the syrphid fly system; however, further empirical evidence of the effects of timing of changes on growth and development in other species remains limited (see Bull *et al.*, 1996; Tenhumberg *et al.*, 2000). Empirical testing of the interaction between long-term changes in food availability and water temperature is now needed to broaden our understanding of how interactions between environmental conditions shape plastic growth and development responses in ectotherms.

Implications for amphibian conservation

Knowledge of how interactions between food availability and temperature influence larval growth, development and survivorship may also be of value to amphibian conservation. Globally, amphibians are declining faster than any other vertebrate group and, for threatened species, the recommended recovery action is captive breeding and reintroduction (Stuart *et al.*, 2004; Gascon *et al.*, 2007). While captive breeding programmes have been established for various amphibian species (Stuart *et al.*, 2004; Gascon *et al.*, 2007), many programmes have been constrained by an inability to generate large numbers of healthy individuals consistently. The ability to generate

large numbers of individuals is critical for three main reasons. First, it allows the captive population to be maintained at a size that avoids problems associated with inbreeding and/or natural attrition. Second, it supplies large numbers of individuals for release, which in various species is a predictor of reintroduction success (Armstrong and Seddon, 2008; Tarszisz *et al.*, 2014). Third, it reduces the cost of captive breeding, making recovery programmes more financially viable (Canessa *et al.*, 2014; Tarszisz *et al.*, 2014). In recognition of the need to improve the productivity of amphibian captive breeding programmes, empirical studies have begun to investigate how anuran growth, development and survivorship are influenced by various abiotic factors, including nutrition (Ogilvy *et al.*, 2012; Dugas *et al.*, 2013; Cothran *et al.*, 2015), pH (Mantellato *et al.*, 2013), salinity (Christy and Dickman, 2002), food availability (Gillespie, 2002) and temperature (Browne and Edwards, 2003). Surprising, however, there remains a limited understanding of how interactive effects between abiotic factors influence anuran life-history traits. Testing for such effects in common model species can be a valuable first step towards identifying optimal rearing environments for threatened species with analogous life histories. For example, by studying the growth and development of the common frog, *Geocrinia rosea*, Mantellato *et al.* (2013) expedited the establishment of *ex situ* breeding programmes for two rare and threatened species, *Geocrinia alba* and *Geocrinia vitellina*.

The aim of the present study was to investigate the independent and interactive effects of long-term exposure to stochastic food availability and water temperature on larval survivorship, growth and development of the striped marsh frog, *Limnodynastes peronii*. To evaluate the effects of food availability and temperature, a 2 × 3 factorial experiment was performed. The 'food availability' factor had two levels, *ad libitum* food supply (constant availability) and stochastic food supply (stochastic availability; Tenhumberg *et al.*, 2000), while the 'temperature' factor had three levels, i.e. 18, 22 and 26°C. The following hypotheses were tested: (i) stochastic food availability would decrease larval survivorship, growth and development; (ii) increased water temperature would increase larval survivorship, growth and development; and (iii) indicative of an interaction between these environmental factors, water temperature would mediate the effects of food availability on survival, growth and development, with decreased food availability having less effect at lower water temperatures due to a lowered metabolic rate, thus requiring less energy to be extracted from the external environment.

Methods

Ethics information

This study was conducted under approval from the University of Wollongong Animal Ethics Committee (Permit Number AE12/23) and the NSW Office of Environment and Heritage (Parks Permit SL101104).

Study species

The striped marsh frog (*L. peronii*) is a common Australian frog species with a wide distribution along the east coast, extending from cool temperate regions in Victoria to the tropical regions of northern Queensland (Wilson, 2001). Larval *L. peronii* are found in various aquatic environments that experience a broad range of nutritional and temperature conditions, making *L. peronii* an ideal model species in which to examine the effects of food availability in combination with temperature variation on larval survivorship, growth and development (Niehaus *et al.*, 2006). The breeding season of *L. peronii* varies depending on geographical location. Within cool-temperate zones, including the Greater Illawarra where the present study was conducted, the breeding season is predominantly late winter through till early summer (Wilson, 2001). Eggs clutches are laid in an aquatic foam nest, and the number of eggs per clutch ranges between 150 and 2000 (Schell and Burgin, 2002).

Clutch collection and tadpole acclimation

Six egg clutches were collected from 25 to 30 January 2013 from a breeding site in the Greater Illawarra region of south-eastern New South Wales (34°26'S 150°51'E). Clutches were collected by hand and stored in separate polyethylene tubs (600 mm × 350 mm × 250 mm) filled with 20 l of Reverse Osmosis water (RO water) and transported to the Ecological Research Centre at the University of Wollongong, Wollongong (34°24'24"S 150°52'46"E). Clutches were maintained in these tubs in natural light conditions at $-25 \pm 2^\circ\text{C}$ for a 10 day acclimation period. This period was imposed to ensure that tadpoles were viable before being entered into the experiment. To ensure no build-up of nitrogenous waste in tubs during the acclimation period, one-third of the water was replaced every fifth day, resulting in two water changes during the acclimation period. Tadpoles hatched from eggs 2–3 days after collection, and once tadpoles had hatched the egg jelly was removed from the tubs and tadpoles were fed *ad libitum* every second day with fish flakes (75% Flora, 25% San; sera GmbH, Heinsberg, Germany).

Approximately 10 days after hatching, tadpoles were entered into experimental housing. Tadpoles were acclimated to the experimental housing for a period of 24 h (see Experimental Design), and any individuals that died during the 24 h were replaced with individuals maintained in identical conditions in order to maintain sample sizes. Tadpoles were fasted during this acclimation period and were provided with food only at the time when they were entered into experimental treatments. Upon entry into the experimental treatments, tadpoles ($n = 48$; split between two replicate rearing tanks per clutch per treatment) were photographed, so that baseline body size measurements could be made at a later date (see Experimental Design). Measurements were not made on back-up replicates, which were euthanased after use. Once tadpoles were entered into the experimental treatments, no further replacement of individual tadpoles occurred.

Experimental design

To examine the effect of temperature and food availability on larval survival, growth and development, a 3×2 factorial design was used. The experiment involved three rearing temperatures (18, 22 and 26°C) and two feeding regimens (constant and stochastic food availabilities), resulting in six experimental treatments referred to as follows: (i) constant 18°C ; (ii) constant 22°C ; (iii) constant 26°C ; (iv) stochastic 18°C ; (v) stochastic 22°C ; and (vi) stochastic 26°C . A split-clutch design was used, with tadpoles from each clutch being randomly allocated to an experimental treatment (i.e. 48 tadpoles per clutch in each treatment split between two replicate plastic rearing tanks, and a total of 288 tadpoles per treatment). Split-clutch designs provide effect controls for both clutch effects and parental genetic effects. The experimental period lasted 14 weeks, and during this time the tadpoles were monitored daily. This experimental period was selected because the larval period in populations of *L. peronii* in southern NSW typically lasts 2–3 months (Anstis, 2013). Furthermore, a past experimental study in *L. peronii* reported that time to metamorphosis in conditions of constant food (*ad libitum* lettuce) and temperature (24°C) ranged between 36 and 55 days (Kraft *et al.*, 2005). The experiment commenced on 12 February 2013 and was terminated on 24 May 2013. During the experimental period, tadpoles were housed in plastic rearing tanks (250 mm × 150 mm × 110 mm). The plastic rearing tanks were rafted within the polyethylene tubs (600 mm × 350 mm × 250 mm), and a Jäger 100 W aquarium water heater (Eheim, Germany) was placed in the polyethylene tub to set the experimental treatment temperature.

A total of 24 tadpoles were housed in each plastic rearing tank at any one time (two replicates of $n = 24$ tadpoles per clutch per treatment); each plastic rearing tank had 2.5 l of RO water, resulting in one tadpole per 105 ml of water. To account for changes to tadpoles per volume of water as a result of tadpole mortality or metamorphosis and to reduce any potential density-dependent effects on growth and development (Miner *et al.*, 2005), 105 ml of water per tadpole was removed to maintain a fixed number of tadpoles per volume of water. These water volume adjustments were carried out on a weekly basis. Experimental tubs were kept in a temperature- and light-controlled room maintained at $12 \pm 2^\circ\text{C}$ ambient temperature and a 12 h–12 h light–dark regimen. To control water salinity, which can have a significant impact on tadpole growth, development and survivorship (see Chinathamby *et al.*, 2006; Kearney *et al.*, 2012), Aquasonic Ocean Nature sea salt was added to the RO water (0.14 g/l). To prevent water fouling, partial water changes (~40%) were made once per week.

The three water temperature treatments (18, 22 and 26°C) in which tadpoles were reared were selected because they reflected the average lower and upper estimates of temperatures that *L. peronii* tadpoles experience in NSW systems during the period between December and April, which is when peak development and metamorphosis typically occurs in this

region (Wilson, 2001). It is of note that *L. peronii* tadpoles in NSW have the capacity to overwinter, and metamorphose from October to November (Anstis, 2013). However, we did not simulate temperatures experienced during this period because, in an effort to make our findings relevant to amphibian captive breeding programmes, we were focused on identifying conditions that promoted rapid larval development without compromising tadpole survival. To ensure that temperatures were maintained at treatment temperatures throughout the entire experimental period, water temperatures were monitored on a weekly basis using a calibrated digital thermometer probe (traceable snap-in module with probe; Thomas Scientific). All treatment temperatures remained within a range of $\pm 2^\circ\text{C}$. To minimize any room effects or tub effects, the temperature of polyethylene tubs was randomly assigned, and plastic tanks were rotated within the polyethylene tub on a daily basis.

Animals were exposed to one of two feeding regimens: constant food availability or stochastic food availability. Constant food availability treatments supplied food *ad libitum* (i.e. no food restrictions applied) throughout the entire experimental period. The stochastic food availability treatment had randomly allocated fasting periods of up to 3 days during which no fresh food was provided. At the start of the fasting period, any uneaten food was removed using a siphon, leaving tadpoles with access to faecal material only. On days when tadpoles had access to food, food was provided *ad libitum* (i.e. no food restrictions applied). *Ad libitum* quantities of food were adjusted throughout the experimental period to account for increased tadpole body size (increased quantities of food) and changes in tadpole density (reduced quantities of food). Food consisted of a mixture of frozen endive (*Cichorium endivia*) and commercial Algae sinking fish pellets (Australian Pet Supplies Feedwell Fishfood, Smithfield, NSW, Australia). To ensure that each plastic tank was treated in the same way, the plastic tanks used for constant food availability treatments also had water siphoned and replaced, and this occurred simultaneous to the beginning of fasting periods for stochastic food availability treatments. This process also assisted in aerating the water.

Effects of food availability and water temperature on survival, development and growth

Survivorship of individual tadpoles in each experimental treatment was monitored on a weekly basis throughout the 14 week experimental period. In addition, for each experimental treatment, the number of tadpoles reaching metamorphosis and the time taken to reach metamorphosis were recorded. Metamorphosis was defined as the time taken for the emergence of at least one forelimb (Gosner Stage 42; Gosner, 1960).

The effects of food availability and water temperature on tadpole size were determined by measuring individual snout-to-vent length (in millimetres). Measurements were made

from digital images acquired on a weekly basis using a standardized overhead digital camera (Canon Powershot D20 12.1 MP CMOS waterproof digital camera). To measure snout-to-vent length, each plastic tank had $\sim 40\%$ of the water removed (coinciding with the partial water change), allowing for enough water to cover the tadpoles but restrict movement within the water column. Snout-to-vent length measurements were made using Image J Image Processing Software (Open Source, version 1.42q), calibrated using a standardized scale present in each photograph. As the tadpoles were housed in groups during the experimental period, tank means, using eight randomly selected tadpoles per tank, were used to preserve data independence. A subsample of eight randomly selected tadpoles was assumed to account for any size variation occurring within each plastic replicate tank (Capellan and Nieceza, 2007).

Within 12 h of the emergence of at least one forelimb (Gosner Stage 42; Gosner, 1960), metamorphs were removed from the experimental treatment container, photographed and maintained in a separate plastic container with an RO water-soaked sponge (3.0 cm²) until the time of tail reabsorption. Prior to tail reabsorption, commercial fish pellets were provided *ad libitum*, and after tail reabsorption pinhead crickets (*Acheta domestica*) were provided *ad libitum*. Containers housing metamorphs were kept in a temperature- and light-controlled room at $22 \pm 2^\circ\text{C}$ ambient temperature under a 12 h–12 h light–dark regimen. Metamorphs were measured within 2 days of tail reabsorption. Vernier callipers were used to measure snout-to-vent length (in millimetres).

Statistical analysis

Effects of food availability and water temperature on tadpole survivorship

To examine the effects of food availability and water temperature on tadpole survivorship over the 14 week experimental period, a Cox proportional hazard model (Andersen and Gill, 1982) was used to determine differences in survivorship distribution; this was displayed as a Kaplan–Meier survival curve. For survival analysis, censorship was applied to death occurring as a result of handling, tadpoles that survived (without metamorphosing) over the experimental period and tadpoles that metamorphosed before the conclusion of the experimental period. To account for any potential clutch effects, clutch identity was included in the model as a random factor. Survival analysis was conducted in the statistical package R v3.1.0 (R Developer Core Team, 2014) in conjunction with the *survival* package (Therneau, 2014).

Survivorship at week 14 was examined further using a generalized linear mixed-effects model (GLMM). In this model, food availability and water temperature were fixed effects and clutch identity was a random effect. The model also included an interaction term between food availability and temperature. Data were analysed in the statistical package R v3.1.0 (R Developer Core Team, 2014) in conjunction with the *survival* package (Therneau, 2014).

Effects of food availability and water temperature on tadpole size

To examine the effects of food availability and water temperature on tadpole size over the 14 week experimental period, a general additive mixed model was used (Lin and Zhang, 1999). The additive model was used because it allows a non-linear growth trajectory in response to experimental treatment (Zuur *et al.*, 2009). Comparisons were made on a weekly basis (weeks 0–9) to examine the additive and interactive effects of food availability and water temperature on tadpole size. To account for any potential clutch effects, clutch identity was included in the model as a random intercept. Tadpole size was measured as snout-to-vent length. Week 0 was used to provide the baseline snout-to-vent measurements, while weeks 1–9 provided the size measurements in response to the experimental treatments. Weeks 10–14 were not included in the general additive mixed model because complete tadpole mortality occurred in several replicates during this period. Data were analysed in the statistical package R v3.1.0 (R Developer Core Team, 2014) using statistical package gamm4 (Wood, 2014).

Effects of food availability and water temperature on development

Over the experimental period, only tadpoles from constant food availability treatments metamorphosed; therefore, all measures relating to metamorphosis were restricted to the constant feeding treatments. To determine the effect of water temperature on the time to metamorphosis and post-metamorphic size, GLMMs were used. In these models, water temperature was the fixed effect and clutch identity was a random effect to account for potential clutch effects. All post-metamorphic data were analysed using the statistical package R v3.1.0 (R Developer Core Team, 2014) using statistical package nlme (Pinheiro *et al.*, 2014).

Results

Effects of food availability and water temperature on tadpole survivorship

Survival of *L. peronii* tadpoles over the 14 week experimental period was significantly different between water temperatures (Cox proportional hazard test: $z = 6.105, P < 0.0001$), but not between food availability treatment groups (Cox proportional hazard test: $z = 0.311, P = 0.760$). There was no significant interaction between food availability and water temperature

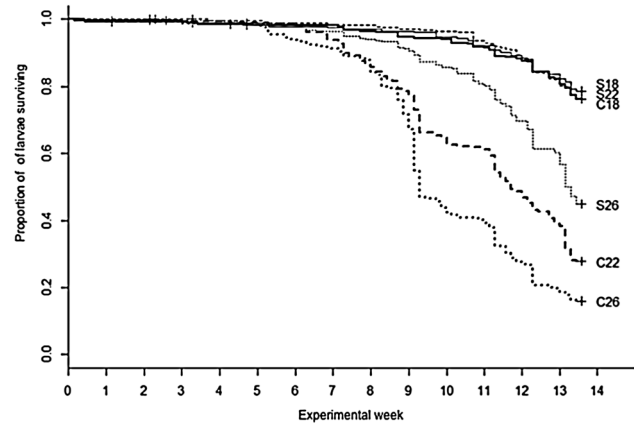


Figure 1: Proportion of *Limnodynastes peronii* tadpoles surviving over a 14 week experimental period in the following six experimental treatments: constant food at 18°C (C18); stochastic food at 18°C (S18); constant food at 22°C (C22); stochastic food at 22°C (S22); constant food at 26°C (C26); and stochastic food at 26°C (S26). '+' indicates a censored event.

(Cox proportional hazard test: $z = -0.843, P = 0.400$; Table 1 and Fig. 1). Survival in all treatment groups was high (>90%) until week 5. After this time, tadpoles began dying in all treatment groups. By experimental week 14, survivorship was lowest in the two warmest constant food treatments (22 and 26°C treatments) and highest in the two coolest stochastic food treatments (18 and 22°C treatments). Survivorship was intermediate in the constant 18°C and stochastic 26°C treatments. Clutch had a significant effect on survival (GLMM: $z = 7.531, P < 0.001$; Tables 2 and 3 and Fig. 2). Whilst there was no overall significant difference between constant and stochastic food availability treatments (GLMM: $z = 1.664, P = 0.0961$; Tables 2 and 3 and Fig. 2), there were significant differences in survival between water temperature treatments, with higher survivorship in the 18°C treatment than in the 22 and 26°C treatments (GLMM: $z = -10.758, P < 0.001$ and $z = -12.943, P < 0.001$, respectively; Tables 2 and 3 and Fig. 2).

Effects of food availability and water temperature on tadpole size

In week 0, there were no significant differences in baseline body size (Table 4). In weeks 1–9, there were significant differences in body size between treatment groups. In week 1, body size was largest in tadpoles from treatments with the warmest

Table 1: Output from Cox proportional hazard model testing the effects of food availability and water temperature on proportion of tadpoles surviving a 14 week experimental period in the striped marsh frog (*Limnodynastes peronii*)

	Coefficient	Exponential (coefficient)	Standard error (coefficient)	Robust SE	z-value	P-value
Stochastic vs. constant	0.5937	1.811	0.5798	1.9113	0.311	0.760
Temperature	0.2392	1.27	0.0146	0.0392	6.105	<0.001
Stochastic: temperature	-0.0742	0.929	0.0246	0.088	-0.843	0.400

Table 2: Effect of food availability and water temperature on percentage of tadpoles surviving to week 14 in the striped marsh frog (*L. peronii*)

Treatment			
Food availability	Temperature (°C)	Sample size	Survival at week 14 (%)
Constant	18	288	73.2 ± 5.2
	22	289	27.5 ± 6.9
	26	289	16.1 ± 5.7
Stochastic	18	287	78.9 ± 5.9
	22	288	77.8 ± 4.7
	26	289	46.1 ± 9.1

Values are means ± SEM. Statistical outputs are from a generalized linear mixed-effects model (see Table 3).

Table 3: Output from general linear mixed-effects model testing the effects of food availability and water temperature on proportion of *L. peronii* tadpoles surviving to week 14

	Estimate	Standard error	z-value	P-value
(Intercept)	1.0547	0.2439	4.324	<0.001
Stochastic vs. constant	0.3362	0.202	1.664	0.0961
Temperature 22 vs. 18°C	-2.0841	0.1937	-10.758	<0.001
Temperature 26 vs. 18°C	-2.7671	0.2138	-12.943	<0.001
Stochastic diet: temperature 22 vs. 18°C	2.0122	0.284	7.085	<0.001
Stochastic diet: temperature 26 vs. 18°C	1.1995	0.2858	4.196	<0.001

water temperatures (22 and 26°C), irrespective of whether food availability was constant or stochastic. In week 2, however tadpole body size was larger in warmest water temperatures (22 and 26°C) and constant food availability treatments (Table 4). In weeks 2–9, body size was largest in treatments with constant food availability, irrespective of the treatment temperature (Table 4). At week 9, a decrease in size with increasing water temperature was evident. An interaction between food availability and water temperature occurred at weeks 3 and 9. Between weeks 0 and 9, there was no effect of clutch identity on tadpole size.

Effects of food availability and water temperature on development

Over the 14 week experimental period, only 2.02% of tadpoles (35 of 1730) reached metamorphosis, and all were from treatments with constant food availability. Of the 35 individuals that successfully metamorphosed, there was no significant effect of temperature or clutch identity on mean time to metamorphosis (in days; Tables 5 and 6) and no effect on post-metamorphic body size (Tables 5 and 6).

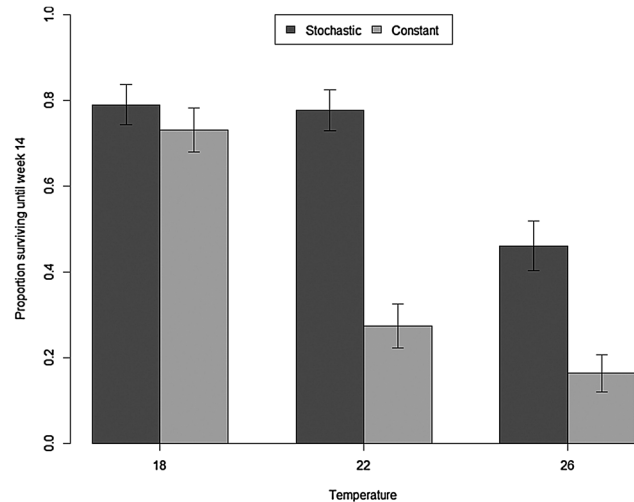


Figure 2: Effect of food availability and water temperature on proportion of striped marsh frog (*L. peronii*) tadpoles surviving to week 14. Stochastic food availability treatments are represented by dark grey bars and constant food availability treatments by light grey bars. Values are shown as means ± SEM.

Table 4: Effect of food availability and water temperature on tadpole size across experimental period weeks 0–9

Week	Water temperature	Food availability	Food availability × Water temperature
0			
1	+		
2	+	–	
3		–	x
4		–	
5		–	
6		–	
7		–	
8		–	
9	–	–	x

Data from weeks 10–14 were excluded because of incomplete sample sizes due to mortality. Positive values (+) indicate a significant ($P < 0.05$) increase in size with increasing water temperature and negative (–) a significant ($P < 0.05$) increase in size with decreasing water temperature. In the case of diet, (–) indicates that the tadpoles with the stochastic diet were significantly ($P < 0.05$) smaller than those with the constant diet. In the interaction term, x indicates a significant interaction between food availability and water temperature occurring. Significance values were derived from the general additive mixed model analysis.

Discussion

The aim of this study was to investigate the independent and interactive effects of food availability and water temperature on larval growth, development and survival in the Striped

Table 5: Effect of food availability and temperature on percentage of tadpoles reaching metamorphosis, time to metamorphosis and post-metamorphic size (snout-to-vent length), in the Striped Marsh frog (*L. peronii*)

Treatment		Percentage metamorphosed ^a		Time to metamorphosis (days)	Post-metamorphic size: snout-to-vent length (mm)
Food availability	Water temperature (°C)				
Constant	18	5.2%	(15/288)	62.1 ± 4.9	15.3 ± 1.9
	22	2.8%	(8/289)	76.4 ± 4.6	15.7 ± 1.5
	26	4.2%	(12/289)	59.4 ± 3.5	14.5 ± 0.7
Stochastic	18		(0/287)		
	22		(0/289)		
	26		(0/289)		

Values are means ± SEM. Note that there are no data presented for the stochastic treatments because no tadpoles in these treatments reached metamorphosis. Sample sizes for post-metamorphic size are $n = 32$. ^aTotal number of tadpoles reaching metamorphosis in each treatment is reported in parentheses.

Table 6: Output from generalized linear mixed effects models testing the effect of water temperature on time to metamorphosis and post-metamorphic size in the striped marsh frog (*L. peronii*)

Time to metamorphosis				
	Estimate	Standard error	t-value	P-value
(Intercept)	4.08631	0.06403	63.82	$<2 \times 10^{-16}$
factor(Temperature)22	0.18496	0.11979	1.544	0.133
factor(Temperature)26	-0.03484	0.09844	-0.354	0.726
Post-metamorphic size				
(Intercept)	2.73954	0.03124	87.687	$<2 \times 10^{-16}$
22 vs. 18°C	0.02102	0.05069	0.415	0.681
26 vs. 18°C	-0.05044	0.03899	-1.294	0.206

Marsh frog, *L. peronii*. Variation in food availability was found to impact larval size and development, with smaller larval size and slower developmental rates in stochastic food availability treatments. Furthermore, changes in food availability mediated the effects of increasing water temperature on survivorship. Specifically, there was smaller larval size and higher survivorship in conditions of stochastic food availability, rejecting our first hypothesis that stochastic food availability would decrease larval survivorship, growth and development. Interestingly, clutch identity did not have a significant effect on any of our measures of tadpole growth and development, but clutch identity did have a significant effect on tadpole survivorship. Given that our clutches were collected over a period of 5 days, it is possible that embryos from different clutches were exposed to different environmental conditions (e.g. pre-treatment temperatures) that subsequently affected their probability of survival. Alternatively, survivorship may have been affected by variable maternal provisioning (Dziminski and Roberts, 2006), differences in parental compatibility (Dziminski et al., 2008) or differences

in parental genetic quality (Sheldon et al., 2003). Such clutch effects have previously been reported in anurans (Sheldon et al., 2003; Dziminski and Roberts, 2006; Dziminski et al., 2008) and underscore the importance of considering the effects of clutch identity in experimental studies aimed at investigating the influence of rearing environment on anuran life-history traits.

The reported effects of food availability on larval growth and development support the predictions of the ‘general optimization model’ (Day and Rowe, 2002), which predicts slower growth and longer developmental periods in stochastic food availability conditions. However, the prediction of a trade-off between growth and development, as described in the Wilbur–Collins model, was not possible to test because the tadpoles did not reach metamorphosis in stochastic food availability conditions (Wilbur and Collins, 1973). Such trade-offs may not have been observed in the present study due to the long-term exposure to stochastic food availability conditions, whereby the threshold sizes or developmental stages were unable to be met. It is probable that the smaller larval size in stochastic food availability conditions resulted in tadpoles being unable to reach this threshold size (to increase developmental rate) within the experimental period. When the experiment was terminated, tadpoles in the stochastic food treatment were still displaying positive growth. Therefore, if the experiment had continued it is possible that tadpoles in these conditions would have reached the minimal size required for metamorphosis, and these individuals would have metamorphosed later and at a smaller size (see Lind et al., 2008). Such a result would have provided support for the Wilbur–Collins model.

Exposure to stochastic food availability conditions throughout development impeded the ability of tadpoles to reach metamorphosis, contrary to the predictions of the ‘stochastic dynamic programming model’, which predicts that larvae respond by pupating earlier and at a smaller size when exposed to changes in food availability throughout development (Tenhumberg et al., 2000). This inability of *L. peronii* to

reach metamorphosis in stochastic food availability conditions suggests that a lack of constant food supply may induce developmental stasis. Developmental stasis may occur due to the inability to reach the threshold size or developmental stage required to increase developmental rates (Wilbur and Collins, 1973). Induced developmental stasis due to changes in food supply has previously been observed in spadefoot toad species (Morey and Reznick, 2000). Spadefoot toad larvae accelerated development in response to restricted food supply, but individuals that had not met the minimal threshold size for development entered developmental stasis (Denver *et al.*, 1998; Morey and Reznick, 2000). An alternative reason why tadpoles did not reach metamorphosis is that tadpoles did not have enough time to reach the developmental threshold required to metamorphose. Tadpoles were still growing when the experiment was terminated, so metamorphosis might have been reached if the experimental period was extended. For this reason, future studies investigating the effect of stochastic food availability on larval growth and development should maintain tadpoles in treatment conditions until either growth rates plateau or tadpoles reach metamorphosis.

According to the temperature-size rule (Kozłowski *et al.*, 2004), growth rate is expected to increase with increasing water temperature because temperature regulates metabolism, growth and differentiation in ectothermic species (Álvarez and Nicieza, 2002b; McLeod *et al.*, 2013). In support of the temperature-size rule and the second hypothesis, the present study shows that *L. peronii* display increased growth with increased water temperature in constant food availability conditions. However, in conditions of stochastic food availability, the effects of water temperature were reduced, suggesting that food availability may restrict the overall energy available for somatic growth processes, thereby preventing significant differences in growth at different water temperatures (Yoneda and Wright, 2005; Inatsuchi *et al.*, 2010; Enriquez-Urzelai *et al.*, 2013).

The slowed growth and developmental rates in conditions of stochastic food availability may have resulted from an overall metabolic downregulation, as predicted by the ‘metabolic downregulation model’ (Keys *et al.*, 1950). Whilst the metabolic rate of larvae was not quantified in this study, lack of food (food restriction) is expected to decrease metabolic costs by limiting growth and development (Wang *et al.*, 2006; Hulbert *et al.*, 2007). The ‘fixed-rate model’ predicts a slower growth rate in conditions of stochastic food availability, with temperature regulating the developmental rate, and cooler temperatures extending the length of the larval period (Travis, 1984). Based on the ‘fixed-rate model’, it would be expected that larvae reared in warmer waters would reach metamorphosis earlier but exhibit a slower growth rate in stochastic food availability conditions. However, long-term exposure to stochastic food availability may reduce energy available for development, preventing metamorphosis, regardless of increased water temperature. Interestingly, there were no differences in time to metamorphosis or post-metamorphic size between water temperature treatments in constant food

availability conditions, which may suggest that the extremes in water temperatures used in the present study may not have differed enough to cause differences in developmental rate. However, this does not seem likely because temperature differences similar to those imposed in this study (18–26°C) have been shown to have drastic effects on the growth and development of various temperate-breeding anuran species (see Blouin and Brown, 2000; Álvarez and Nicieza, 2002a, b; Browne and Edwards, 2003; Walsh *et al.*, 2008).

As mortality in the juvenile life stages of amphibians is typically high (Canessa *et al.*, 2014), it can be difficult to make generalizations about the effects of experimental treatments on growth, development and survivorship. However, experimental studies are still useful for making inferences about treatment effects (e.g. Kearney *et al.*, 2012, 2014). It was found that survivorship decreased in the warmest temperature treatments and stochastic food availability conditions buffered against mortality losses at higher temperatures. Warmer waters may have compromised survival because of decreased oxygen availability (O’Connor *et al.*, 2007; Blaustein *et al.*, 2010), oxidative stress due to higher metabolic rate (Hulbert *et al.*, 2007), the build-up of microbes from decomposing food (McWilliams, 2008) or nitrogenous waste products (Morey and Reznick, 2004) or changes in the intensity of competition (Álvarez and Nicieza, 2002b; Blaustein *et al.*, 2010; Enriquez-Urzelai *et al.*, 2013; McLeod *et al.*, 2013). A previous study examining the effects of long-term changes to food availability and water temperature on coral fish species also reported low survivorship in warmer waters and suggested that survival may have been compromised due to starvation. Consequently, when exposed to high food availability conditions, coral fish survivorship increased (McLeod *et al.*, 2013). Conversely, in the present study, the long-term stochastic food availability treatment reduced the effects of the warmest water temperatures, with higher survivorship in stochastic food availability treatments. Slower growth rate as a result of stochastic food availability conditions may reduce the effects of water temperature on survivorship in larval *L. peronii*. As a result, growth conditions may influence the risk of mortality (Enriquez-Urzelai *et al.*, 2013).

A number of studies have investigated the impacts of changes in food availability (or quality) interacting with other environmental factors and have documented induced changes in growth and development; these include interactions between food quality and temperature (Álvarez and Nicieza, 2002b), between predation risk and food availability (Nicieza, 2000) and between pond desiccation and food availability (Enriquez-Urzelai *et al.*, 2013). The interactions between environmental factors can be varied, with the interactive effects also being difficult to predict (Álvarez and Nicieza, 2002b). For example, in a study investigating the effects of water temperature and food quality on growth and development in Iberian painted frogs (*D. galganoi*), it was found that water temperature had persistent effects on development and metamorphic traits, with larvae metamorphosing later and at larger body size when reared at lower temperatures. However, the effects of food

quality on growth and development were largely dependent on water temperature, with larvae fed carbohydrate-rich diets being smaller at metamorphosis compared with larvae fed protein-rich diets, but not at all water temperatures (Álvarez and Nicieza, 2002b). Conversely, Enriquez-Urzelai *et al.* (2013) investigated the interactive effects between food availability and desiccation on the painted frog (*Discoglossus pictus*), observing that size and weight at metamorphosis were determined by food availability but not by the water desiccation regimen. The results of the present study strongly suggest that environmental differences in food availability and water temperature, and their interaction, cause differences in growth, development and survivorship. In this experiment, it was determined that food availability was more important than water temperature for survivorship, growth and development; a pattern that has also been described in larval coral fish species (McLeod *et al.*, 2013).

Implications for amphibian conservation

Our finding that changes in food availability mediate the effects of temperature on *L. peronii* growth, development and survivorship has implications for amphibian conservation. Similar to *L. peronii*, many threatened anurans are temperate-zone pond-breeding species, in which larvae experience marked fluctuations in temperature and food availability over extended developmental periods (i.e. >2 months). Captive breeding programmes attempting to breed such species generally rear tadpoles in constant environmental conditions, but our findings suggest that managers might benefit from manipulating both food availability and temperature. Specifically, providing individuals with stochastic food availability at warmer temperatures may improve individual survivorship and the likelihood of generating large numbers of tadpoles. This could benefit the recovery of a target species by improving the sustainability of a captive ‘insurance’ population, whilst minimizing expenses associated with establishing and maintaining colonies (Canessa *et al.*, 2014). Furthermore, generating large numbers of individuals for release could improve the success of reintroduction programmes by ensuring the release of large groups, which could overcome problems associated with high dispersal, demographic stochasticity or low reproduction and/or survival at low population densities (Allee effects; Fischer and Lindermayer, 2000; Armstrong and Seddon, 2008). For these reasons, we propose that broadening our knowledge of the effects of interactions between environmental factors on anuran growth, development and survivorship might improve the success of management of threatened amphibian species (Carey, 2005; Muths *et al.*, 2014).

Conclusions

In conclusion, the aim of this study was to use a manipulative laboratory experiment to examine the independent and interactive effects of long-term stochastic food availability and water temperature on larval *L. peronii* survivorship, growth and development. Larval growth rate was highest and survivorship lowest at the warmest temperature. However,

changes in food availability mediated the effects of temperature, with slower larval growth and higher survivorship in stochastic food availability treatments. These findings contribute to a small but growing body of evidence that interactions between environmental factors can influence anuran growth, development and survivorship. Such advances have the potential to improve the output of amphibian captive breeding programmes and aid amphibian conservation.

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