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Poor Nutritional Conditions During the Early Larval Stage Reduce Risk-Taking Activities of Fire Salamander Larvae (*Salamandra salamandra*)

E. Tobias Krause, Sebastian Steinfartz & Barbara A. Caspers

Department of Animal Behaviour, Bielefeld University, Bielefeld, Germany

Correspondence
E. Tobias Krause, Department of Animal Behaviour, Bielefeld University, PO Box 100 131, 33501 Bielefeld, Germany.
E-mail: tobias.krause@uni-bielefeld.de

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Abstract

Environmental conditions experienced early in life have been shown to significantly affect growth trajectories at later stages in many vertebrate species. Amphibians typically have a biphasic life history, with an aquatic larval phase during early development and a subsequent terrestrial adult phase after completed metamorphosis. Thus, the early conditions have an especially strong impact on the future survival and fitness of amphibians. We studied whether early nutritional conditions affect the behavioural reaction of fire salamander larvae (*Salamandra salamandra*) before completion of metamorphosis. Fire salamander larvae reared under rich nutritional conditions were heavier and larger, displayed better body condition overall throughout the first three month of life and metamorphosed earlier compared with larvae raised under poor nutritional conditions. Specifically, we tested whether larvae reared under these different conditions differed with respect to their risk-taking behaviour and activity. We found no differences in the activity of larvae with respect to their experienced early food conditions. However, larvae reared under poor nutritional conditions hid significantly more often in a risk-taking test than larvae reared under rich food conditions. This increase in shelter-seeking behaviour might be an adaptation to reduce the risk of larval drift or an adaptation to compensate for physiological deficits in part by appropriate behavioural reactions. Our results indicate that environmental conditions, such as food availability, may lead to different behavioural strategies.

Introduction

Phenotypic variance among individuals at the population level can affect future individual fitness. In general, different individual phenotypes result from the interaction between individual genotypes and environmental conditions experienced throughout life, especially during the earliest phases (Monaghan 2008). Environmental conditions experienced early in life have been shown to affect growth trajectories in many vertebrate species (Lindström 1999). The environmental structure (Fox & Millam 2004; Krause et al. 2006), population density (Eitam et al. 2005), presence of predators (Van Buskirk & Relyea 1998; Van Buskirk & McCollum 2000), parasite load (Bize et al. 2003) and food availability (Walling et al. 2007; Krause et al. 2009; Warburg 2009; Honarmand et al. 2010) are known as crucial factors in the early environmental conditions experienced by an individual. Amphibians typically have a biphasic life history with an aquatic larval stage during early development and a subsequent adult phase after the completion of metamorphosis. Thus, early conditions can have a strong impact on future performance (Wilbur

& Collins 1973; Semlitsch et al. 1988; Ryan & Semlitsch 2003). Most species of amphibians hatch from eggs, but salamander species representing the group of 'fire salamanders' (see Steinfartz et al. (2000) for a systematic overview) are lecithotrophic larviparous (see Greven 1998), i.e. give birth to larvae that are fed from their egg capsules, which the female deposits in small streams where they grow and develop until metamorphosis (Freytag 1955; Thiesmeier 2004). Fire salamanders, however, do not rely entirely on small streams as larval habitats; populations of some species also use temporary and stagnant water bodies such as ponds or little ditches, which are ecologically very different from small streams. Most importantly, pond larvae show specific adaptations to deal with the lower food supply and higher desiccation risk experienced in their habitats compared with small stream habitats (see Weitere et al. 2004; Steinfartz et al. 2007). After metamorphosis, fire salamanders are completely terrestrial, only returning to the water for the deposition of larvae. Accordingly, during the larval phase, fire salamander larvae can be exposed to stressful periods (Alcobendas et al. 2004; Eitam et al. 2005; Warburg 2009). Generally, growth, survival and age at metamorphosis in amphibians are affected by various abiotic as well as biotic environmental factors, such as water temperature, food availability, larval density and cohort effects (Wilbur & Collins 1973; Werner 1988; Day & Rowe 2002; Alcobendas et al. 2004; Weitere et al. 2004; Eitam et al. 2005; Warburg 2009).

Food availability has been shown to be one of the most crucial factors during the early life phase of an individual (Metcalfe & Monaghan 2001; Monaghan 2008). In zebra finches for example, poor food conditions experienced during an early stage of life affected body resource management and resulted in different foraging and risk-taking behaviour at later life stages compared with normally nourished individuals (Krause et al. 2009).

In this study, we investigated whether early nutritional conditions experienced by fire salamander larvae affect their risk-taking behaviour, i.e. the shelter-seeking behaviour. The shelter-seeking behaviour in amphibian larvae can be induced by the presence of potential predators, indicating that hiding under a shelter reduces the chance of being discovered by a predator (Werner & Anholt 1993). Because larvae in poor physical condition likely have a higher risk of predation than larvae in good body condition, we expect larvae reared under early nutritional stress conditions to show similar behavioural adaptations to those resulting from predator presence during early development. We propose that larvae from poor food conditions exhibit decreased body condition and increased shelter-seeking behaviour compared with those from rich early food conditions.

Methods

Study Animals

For the experiments, we used 24 larval fire salamanders (i.e. 12 sibling pairs) that were deposited by wild-caught fire salamander females in the laboratory. Pregnant females of *Salamandra salamandra* were collected in the spring of 2010 from the Kottenforst in Germany (50°41′09N, 7°07′03E). Females deposited larvae from Mar. 2010 to May 2010 in the laboratory of the Department of Animal Behaviour at Bielefeld University. From each of the 12 mothers, we used a random pair of siblings and tested one larva from each pair under poor nutritional treatment conditions (hereafter called *poor conditions)* and the other larva under rich food conditions (hereafter called *rich conditions*). Larvae were weighed with a Sartorius BL150S balance (accuracy $\pm$ 0.001 g), and total body length was measured using a plastic-coated millimetre paper (accuracy $\pm$ 0.5 mm) at the day of birth and subsequently after 30, 60 and 90 d. A body condition index (body mass [g]/body length [mm]; after Karraker & Welsh 2006; Stevenson & Woods 2006) was calculated for each larva at different stages (i.e. after 30, 60 and 90 d after deposition), and we recorded the age in days after deposition at metamorphosis. Survival through the duration of the nutritional treatments was also recorded.

Nutritional Treatments

We experimentally manipulated the nutritional conditions during the first 3 mo (i.e. 90 d after deposition) of life, and thus, our nutritional treatments almost covered the complete larval phase of fire salamanders, as is typically experienced by *Salamandra salamandra* in Central European habitats (Thiesmeier 2004). During the larval stage, individuals were kept separately in water-filled plastic cups (diameter 8 cm, height 9 cm) that were housed in a climate chamber at constant temperature (night/day: 11/14°C) and light conditions (dark/light cycle: 13/11 h). At the age of 2 mo, each larva was transferred into a small plastic tank (length 20 cm, width 10 cm, height 9 cm) filled with water and additionally partly bedded with soil (approximately one-third of the tank) to guarantee that larvae were able to leave the water after metamorphosis. Tanks were stored in a conditioned room (dark/light cycle: 10/14; temperature: 20.5°C).

Larvae in the *poor*-condition treatment group received food twice a week, while larvae in the *rich*-condition treatment group received food six times a week. The amount of food given to each larva was the same at each feeding. We initially started feeding three living and similarly sized *Chironimus* larvae and increased their number by one every other week.

Testing for Risk-Taking Behaviour of Larvae

We tested the risk-taking behaviour of each larva at the age of 1 and 2 mo. At the start of each trial, the larva was placed in a water-filled Petri dish (diameter 8.7 cm). Half of the dish was covered with an opaque plastic shield, while the other half was left uncovered. Each larva was released through a predefined opening in the centre of the uncovered part. During the 120-s test, we recorded activity as the number of shifts between the uncovered and covered area. Additionally, we measured the time each larva spent in the uncovered and covered area as a measurement of risk taking.

The experiments were carried out according to the German Laws for experimentation with animals. All salamanders were collected from the field and maintained during the course of the experiments with respective permissions from the 'Untere Landschaftsbehörde der Stadt Bonn'.

Statistical Analysis

Physiological parameters such as body length, body mass and condition index were analysed using linear mixed effect models (LME) with nutritional treatment as factor and mother identity as random factor. Behavioural data, risk-taking activity and metamorphosis-related data were analysed with a Wilcoxon signed-rank test for the sibling pairs. Survival was analysed with a chi-square test. All analyses were performed with implemented test options of the program SPSS 18 (SPSS Inc., Chicago, IL, USA).

Results

Physiological/Morphological Parameters

When deposited by their mothers, all larvae assigned subsequently to the different experimental treatments did not differ in their total body length, body mass (see Table 1a,b) or condition index (LME, treatment $F_{1,11} = 2.83$, $p = 0.12$). At the age of 1 mo (i.e. 30 d), individuals treated under *rich* conditions were significantly larger and heavier than individuals from the *poor*-condition group (see Table 1a,b), which was also reflected by significantly higher condition indices (LME, treatment $F_{1,11.00} = 63.57$, $p < 0.0001$). At the age of 2 mo (i.e. 60 d), larvae from *rich* conditions were still significantly larger and heavier than larvae that experienced *poor* conditions (see Table 1a,b) and also had higher condition indices (LME, treatment $F_{1,10.22} = 140.60$, $p < 0.0001$). These differences were still present at the age of 3 mo, where most individuals were already metamorphosed (Table 1a,b. condition index: LME, treatment $F_{1,10.72} = 32.20$, $p = 0.0002$). Larvae that experienced *poor* conditions metamorphosed significantly later (on average 20 d later) than individuals from *rich* conditions (Wilcoxon $N = 10$, $Z = -2.81$, $p = 0.005$; range from 61 to 110 d; Fig. 1). In total, two larvae died during the first 3 mo. Both of them were kept under *rich* conditions, but larval mortality was not statistically different

Table 1: The a) mean body length of the larvae and b) mean body masses of the young fire salamander ($\bar{x} \pm$ SD) in the two nutritional treatments (poor and rich) at four different age stages (birth, 1, 2 & 3 mo) are shown

	Poor treatment	Rich treatment	df	*F*	p
a) Body length in mm					
at birth	31.23 ± 2.77	31.11 ± 2.80	1,11	0.19	0.67
at 1 mo	35.31 ± 3.16	38.17 ± 3.99	1,11	57.51	<0.0001
at 2 mo	38.26 ± 3.22	46.03 ± 3.61	1,9.2	152.77	<0.0001
at 3 mo	39.85 ± 2.11	47.13 ± 2.68	1,9.6	83.9	<0.0001
b) Body mass in g					
at birth	0.198 ± 0.06	0.186 ± 0.05	1,11	2.75	0.13
at 1 mo	0.267 ± 0.06	0.389 ± 0.12	1,11	49.39	<0.0001
at 2 mo	0.366 ± 0.07	0.713 ± 0.14	1,10.2	130.61	<0.0001
at 3 mo	0.467 ± 0.08	0.719 ± 0.10	1,10.46	52.76	<0.0001

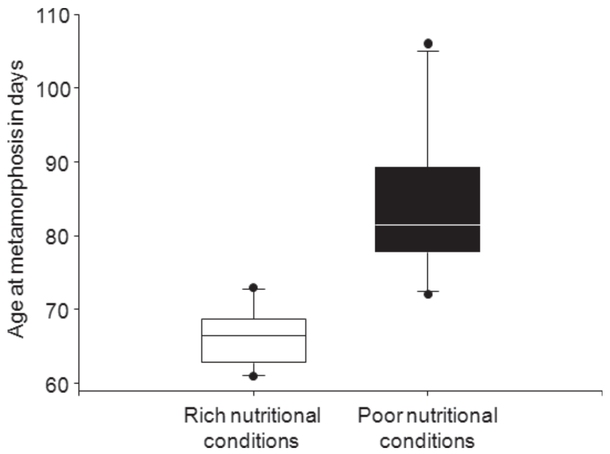

Fig. 1: Age at metamorphosis from larvae of the two different (*poor* and *rich*) early nutritional treatments. The larvae from *rich* treatment metamorphosed significantly earlier than the larvae from *poor* treatment (p = 0.005, see text for details). The central line in the boxes indicates the median, and the spread of the box indicates the 25% and 75% quartile. The whiskers indicate the entire data range. Outliers are indicated by dots.

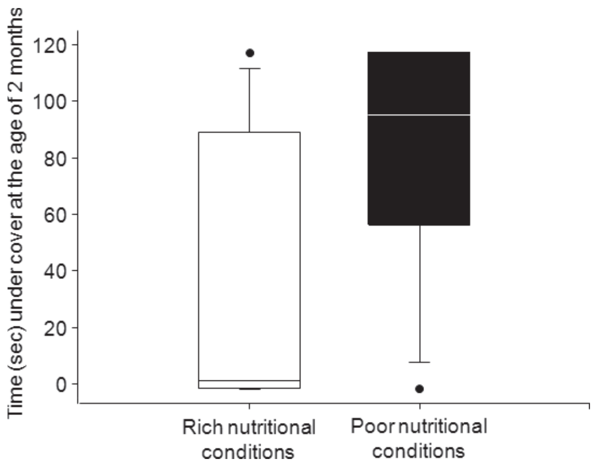

Fig. 2: Time (in seconds) fire salamander larvae spent under cover in the risk-taking test at the age of 60 d. Individuals from *poor* nutritional treatment spent significantly more time sheltered under cover (p = 0.012, see text for details). The central line in the boxes indicates the median, and the spread of the box indicates the 25% and 75% quartile. The whiskers indicate the entire range. Outliers are indicated by dots.

between the two treatment groups (Chi-square-test, df = 1, $\chi^2 = 2.18$, p = 0.14).

Behavioural Tests

There was no difference in the risk-taking behaviour of larvae kept under *poor* and *rich* conditions at the 1-mo stage. Larvae of both treatments spent similar amounts of time in the uncovered and covered areas (Wilcoxon, $N = 12$, $Z = -1.43$, p = 0.16). At the age of 2 mo, however, larvae that experienced *poor* conditions spent significantly more time under the covered area than those kept under *rich* conditions (Wilcoxon, $N = 11$, $Z = -2.50$, p = 0.012; Fig. 2).

The activity parameter, measured as the number of shifts between the uncovered and covered area in the risk-taking test, was not significantly different between the two treatment groups at the stage of 1 mo (Wilcoxon, $N = 12$, $Z = -0.85$, p = 0.39) or 2 mo (Wilcoxon, $N = 11$, $Z = -0.31$, p = 0.76).

Discussion

As expected from a broad body of literature investigating metamorphic timing of amphibian larvae, body growth, overall body condition and time until metamorphosis in fire salamander larvae were strongly impacted by early food conditions (Denoël & Poncin 2001; Thiesmeier 2004; Weitere et al. 2004; Warburg 2009). Our data show that larvae of *Salamandra salamandra* reared under the rich nutritional conditions were heavier, larger and in better overall condition throughout the first 3 mo of life than larvae raised under the poor nutritional conditions. Survival was not affected by nutritional conditions, but individuals from the rich nutritional conditions completed the metamorphosis up to 20 d earlier. Furthermore, we showed that early nutritional conditions in fire salamander larvae influenced risk-taking behaviour, i.e. shelter-seeking behaviour.

Several studies have shown the effect of environmental factors during the larval stage on physiological and morphological development in fire salamander larvae. For example, growth, survival, age at metamorphosis and metamorphic timing of fire salamander larvae are influenced by environmental factors, such as temperature, availability of food, density of conspecifics and cohort effects (Denoël & Poncin 2001; Alcobendas et al. 2004; Thiesmeier 2004; Weitere et al. 2004; Eitam et al. 2005; Warburg 2009). However, few studies have addressed the possible behavioural effects because of experienced environmental conditions during the early larval stage. Our current results reveal that such effects on behaviour exist. Observed behavioural differences were not present at the beginning of our nutritional treatments but developed at a later larval stage (i.e. after 60 d of larval development) as a consequence from experienced poor nutritional conditions during the early larval period.

Increased 'shelter seeking' in amphibian larvae has only been observed in the context of larval defence mechanisms induced by the presence of potential predators thus far (Van Buskirk & Relyea 1998; Van Buskirk & McCollum 2000; Mathis et al. 2003). The presence of potential predators can cause a cascade of species-specific behavioural and morphological responses in amphibian larvae, resulting in reduced activity, increased tendency to hide and reduced growth and development, known as the growth/predation risk trade-off (McPeek 2004). Interestingly, fire salamander larvae kept under poor nutritional conditions show behavioural traits that are typical for predator-induced defence. The influence of predators can be ruled out in our experiment, and thus, poor early food conditions alone must have caused a similar response. It is known that the behavioural changes induced by the presence of a predator cannot directly explain the reduced growth and development (McPeek 2004). Our results, however, show that reduced growth and development can induce behavioural changes. It seems that stressful periods in the early life of amphibians induce behavioural adaptations, which can be triggered by different mechanisms.

Another explanation may be an adaptation to reduce the danger of larval drift downstream, which poses a significant threat to larvae of *Salamandra salamandra* under natural conditions as larvae will be transported into areas where they will be preyed on by fish species (Thiesmeier & Schuhmacher 1990). It has been shown that small larvae are more affected by drift than larger ones (Thiesmeier & Schuhmacher 1990; Thiesmeier 2004). Increased shelter-seeking behaviour of small larvae (in our case represented by larvae from poor nutritional conditions) might be an adaptive strategy to reduce the risk of drift under natural conditions. Because hiding places under flat stones or fallen leaves in the streams provide sites with a reduced risk of drift. Behaviour thus offers a possible mechanism to fire salamander larvae that experience poor nutritional conditions to cope with and adapt to such a situation. Our results indicate that early environmental conditions lead to important alternations on the behavioural level, which can have fitness relevant consequences for later developmental stages, which may be generalised to any stress during early life.

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