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ORIGINAL ARTICLE

Food level and light conditions affect the antipredator behavior in larvae of a stream-breeding amphibian

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Abstract

Understanding how long-term changes in environmental conditions influence the way that individuals cope with threats is essential in the context of behavioral adaptation to a rapidly changing world. However, little is known about the behavioral responses to predation risk for individuals that experienced different environmental conditions for extended periods of time, such as food levels and light conditions. In this experimental study, we tested whether previous long-term exposure to different food levels (low versus high) and light conditions (0-h light versus 8-h light) plays a significant role in shaping the antipredator response (i.e., the probability of emerging from the refuge and the distance moved) to stimuli from caged larval dragonflies, in larvae of the fire salamander (*Salamandra salamandra*). Specifically, we quantified behavioral differences in the response to predation risk in larval salamanders that were reared in the laboratory for 2 months under controlled food and light conditions. The results of this study showed that the interaction between food level and light conditions affected the antipredator behavior of the larvae. Fire salamander larvae maintained at low food levels and in 8-h light conditions emerged from the refuge with a higher probability (i.e., took more risk) than larvae maintained at high food levels and all other combinations of light conditions. Thus, our results highlight the complexity of antipredator responses, pointing attention to the fact that interactions among environmental factors are likely to determine the magnitude of antipredator response.

Significance statement

Few studies have investigated the role of multiple environmental factors on the expression of predator-induced behavioral responses. Specifically, because no study has so far investigated the risk-taking behavior in individuals exposed to long-term, contrasting food levels, and light conditions, we investigated this in amphibian larvae. We showed that environmental conditions interactively determined antipredator behavior. This highlights the importance of considering long-term environmental conditions experienced by an organism and their interactions when experimentally studying behavioral variation to adequately understand its expression in natural conditions.

Keywords Behavioral response · Environmental cues · Food levels · Refuge emergence · Light condition · Salamander

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Introduction

The antipredator behavior of a prey is determined by the trade-off between predator avoidance and obtaining resources (Sih 1980; Werner and Anholt 1993; Lima 1998). While predator-induced changes in behavior, morphology, and life history increase prey survival, these changes also have fitness costs. Reduced prey foraging activity in the presence of predators has knock-on effects on important fitness components such as growth and investment in reproduction (Cressler et al. 2010). Thus, prey trade-off predation avoidance and feeding activities such that fitness is maximized. Theoretical and empirical studies have been shown that the time allocated to these activities is heavily dependent on the physiological state of individuals and environmental conditions (Lima and Dill 1990; Semlitsch and Reyer 1992). Food availability is an environmental condition which is well known to affect the trade-off between foraging and predator avoidance (Anholt and Werner 1995). For a variety of prey species, it has been demonstrated that the antipredator responses are mediated by food availability and feeding history of individuals (Lima and Dill 1990; Lima 1998). For example, satiated individuals take less risk than food-deprived individuals, with many examples from birds (Heller and Milinski 1979; Tvardíková and Fuchs 2011; Bonter et al. 2013), fish (Godin and Crossman 1994; Sogard and Olla 1996; Poulsen et al. 2010), and insects (Nonacs and Dill 1990). Food level also affects the antipredator behavior of amphibian larvae (Horat and Semlitsch 1994; Anholt et al. 1996; Anholt et al. 2000; Whitham and Mathis 2000; Altwegg 2003; Carlson et al. 2015; Kurali et al. 2018). Although food availability and its effect on behavioral antipredator responses is well documented, there is a lack of studies investigating how other environmental factors may further interact with feeding history to shape threat-sensitive responses of animals.

The light/dark cycle is a major environmental factor that influences the physiology and the behavior of most organisms (Albrecht and Oster 2001). This rhythm is known to have important effects on animal body temperature (Erskine and Hutchison 1982), physiological performance (e.g., tolerance to higher temperatures) (Hutchison et al. 1979), food intake (Zhou et al. 1998), activity patterns (e.g., vertical migration exhibited by salamanders; Anderson and Graham 1967), and a variety of other behaviors (e.g., reproductive, antagonistic and antipredator behavior) (Rand et al. 1997; Wise and Jaeger 1998; da Silva Nunes 1988). Physiological consequences are especially common for amphibians, for which changes in the lighting conditions affect the production of melatonin (Gern et al. 1983), a hormone responsible for many aspects of the photoperiodic physiology and behavior (Vanecek 1998). In salamanders, Wise and Buchanan (2006) suggested that lower levels of nocturnal plasma melatonin caused by a longer photophase increase the metabolic rates, which in turn increase the energy demands.

Thus, we expect that light conditions and food availability interactively determine the behavioral responses of amphibians to predation risk. Previous studies showed that light conditions influence foraging and antipredator behavior in amphibians (Taylor 1984; da Silva Nunes 1988; Ding et al. 2014). Among the various parameters of light that can affect amphibian behavior, light intensity appears to be the most important one. For instance, Panamanian cross-banded tree frogs (*Smilisca sila*) were found to exhibit changes in the antipredator behavior depending on illumination level (da Silva Nunes 1988). Light intensity has also been shown to play a key role in amphibian predator-prey interactions, for example the likelihood to encounter diurnal or nocturnal predators (Taylor 1984). However, day length acts as an environmental cue to which individuals respond by developing behavioral strategies (Ding et al. 2014). Variation of light exposure over a short- and long-term duration may significantly alter the energy demands and the threat-sensitive behavior. Circadian and seasonal patterns of activity are adaptive behavioral traits which allow individuals to effectively exploit available resources.

Light conditions are expected to have marked influence on species with activity patterns regulated by the photoperiod, for instance salamanders (Wise and Buchanan 2006). The European fire salamander (*Salamandra salamandra*) is one of the salamander species which is able to exploit both surface and underground habitats for breeding (Manenti et al. 2011). Fire salamanders present high behavioral plasticity which allows them to colonize underground habitats (Manenti et al. 2013, 2016), a challenging environment due to its unique characteristics, such as low food availability and perpetual darkness. Thus, fire salamanders are ideal organisms for testing the interaction of these environmental variables on antipredator behavior in prey species. The aim of our study was to investigate the interactive effects of the long-term exposure to contrasting food levels and light conditions on the antipredator response in larvae of the fire salamander (*Salamandra salamandra*). We used the probability to emerge from the refuge and the distance moved outside the refuge as antipredator responses describing risk-taking behavior (Sih et al. 1988; Anholt and Werner 1995; Oswald et al. 2020). Specifically, we hypothesized that long-term exposure to low food levels coupled with longer photoperiod would decrease antipredator response in prey (i.e., larvae will emerge from the refuge more frequently and travel longer distances) when exposed to cues from a predator.

Material and methods

Sampling and housing conditions

Fire salamanders give birth to fully aquatic larvae in both surface and underground sites (Manenti et al. 2013). We

collected 96 newborn larvae of *S. salamandra* in April 2017 from three sites in the Romanian Carpathians. Two sites were located underground: inside an artificial subterranean habitat (Buzau, 45.4° N, 26.2° E, 475 m elevation a.s.l., $n = 23$) and inside a cave (Gaura cu Musca Cave, 44.6° N, 21.6° E, 92 m elevation a.s.l., $n = 25$). The third site was above the surface: a small reservoir along a forest stream (Iconie, 44.9° N, 22.7° E, 325 m elevation a.s.l., $n = 48$). We chose three populations to account for potential variation in behavior, while assuring a large sample size to test for multiple experimental factors. In the underground sites, the European cave spider *Meta menardi* is likely to prey on salamander larvae. In surface sites, several dragonfly species (Gomphidae, Anisoptera) coexist with and predate on salamander larvae.

The experiment was conducted in the Laboratory Gallery of Closani Cave (air temperature 13 ± 1 °C), which is the cave-laboratory of "Emil Racoviță" Institute of Speleology, located in Gorj county, southwestern Romania. All collected larvae corresponded to the developmental stage 1 which was recognizable by a well-developed and bluntly rounded tail-fin (Juszczyk and Zakrzewski 1981). The mean ± standard error of the snout-vent length (SVL) of the salamander larvae at the beginning of the experiment (hereafter SVL initial) was 18.9 ± 0.09 mm.

In the laboratory, we allocated the 96 salamander larvae at random, but we assured a balanced number of larvae having different origins (i.e., surface, $n = 48$; underground, $n = 48$) in each of the four experimental treatments representing a combination of two food levels (low versus high) and two light conditions (0-h light versus 8-h light). Since SVL initial differed significantly among populations ($F_{[2,\ 90]} = 6.185$, $P = 0.003$; Tables S1 and 2 in the Supplementary Material), we allocated the larvae to the experimental treatments in such a way to assure that larvae did not differ in their SVL initial either between food ($F_{[1,90]} = 0.141$, $P = 0.708$) or light ($F_{[1,\ 90]} = 0.166$, $P = 0.685$) treatments (Table S1 in the Supplementary Material). There were 24 larvae per treatment. Larvae were kept individually in plastic containers (length × width × height: 21 × 14 × 9.5 cm; 2 cm of water depth). This water depth is within the range of habitats found in the wild and follows previous standardized design (Manenti et al. 2013). Larvae in the 8-h light treatment were reared at a light intensity of 50 lux measured above the rearing containers (the source light was placed on the ceiling of the Laboratory Gallery of Closani Cave). Larvae were fed live worms (*Tubifex* sp.) every third day, and the water was changed the day after feeding. The larvae in high food treatments were fed six live prey items per feeding throughout the first 3 weeks and ten prey items per feeding from the fourth week. The larvae in low food treatments received half the amount of food compared to larvae in the high food treatments. These food levels were established based on those used in similar experiments involving fire salamander larvae (Manenti et al. 2013).

Behavioral experiments

Observations of larval fire salamander behavior were conducted after 2 months of maintaining the larvae in the four treatments described above. The 2-month threshold was chosen because this amount of time was sufficient for the experimental treatments to have a significant effect on the body size of fire salamander larvae (Krause et al. 2011) (Tables S1 and 2 in the Supplementary Material). We also considered 2 months of experimentally manipulated food levels and light conditions to cover more than half of a typical larval phase of fire salamanders (Thiesmeier 2004). Our experimental setup followed a 2 × 2 × 2 repeated-measures design whereby the fire salamander larvae maintained under the two food levels (low versus high) and two light conditions (0-h light versus 8-h light) were subsequently observed during two successive scenarios: no risk (i.e., during the absence of a predatory stimulus), and risk (i.e., during the exposure to a predatory stimulus).

The behavioral observations were conducted in white opaque plastic containers (l × w × h: 30.5 × 21.5 × 14.8 cm, 2 cm of water depth). We used opaque containers to visually isolate the salamander larvae. Each container was divided in two distinct zones: a refuge (6.5 × 21.5 × 14.8 cm) and an open arena (25 × 21.5 × 14.8 cm). The refuge was located at one end of the container and had a chamber-like construction, being separated from the arena by an opaque plastic sliding door, while the top of the refuge partition was covered with a removable opaque plastic lid (Fig. 1). The arena was represented by the remaining open space in the container and served for the observation of the behavior (Figs. 1 and 2). Prior to the onset of each behavioral observation, in the no risk scenario, we placed an empty white, plastic-framed cage (h × diameter: 6.4 × 3.5 cm) covered by transparent mesh (mesh size = 0.2 cm), in one corner of the arena (opposed to the refuge). In the risk scenario, we placed the framed mesh cage in the same way but with two live dragonfly larvae inside. We used in total eight Gomphidae (Anisoptera) dragonfly larvae of similar sizes (mean length ± SD: 18.4 ± 2.7 mm). We placed the same two dragonfly larvae in one cage but the caged dragonfly pairs were randomly assigned during the behavioral observations. We collected the dragonfly larvae from Bulba River, which is located in proximity to the surface population of salamanders. The dragonfly larvae were collected the night before we started the behavioral experiment. The dragonfly larvae were not fed before or among behavioral observations, and after the experiment, they were released at the collecting site. Previous studies indicated that salamanders rely on chemical cues for predator recognition (Cupp 1994) and that chemical alarm cues obtained from dragonfly larvae of Anisoptera elicited strong antipredator response in larval fire salamanders (Manenti et al. 2016). In both risk scenarios, a transparent mesh bag (l × w: 8.5 × 7 cm) with 10 live *Tubifex* sp. was placed in the other corner of the arena. The position of

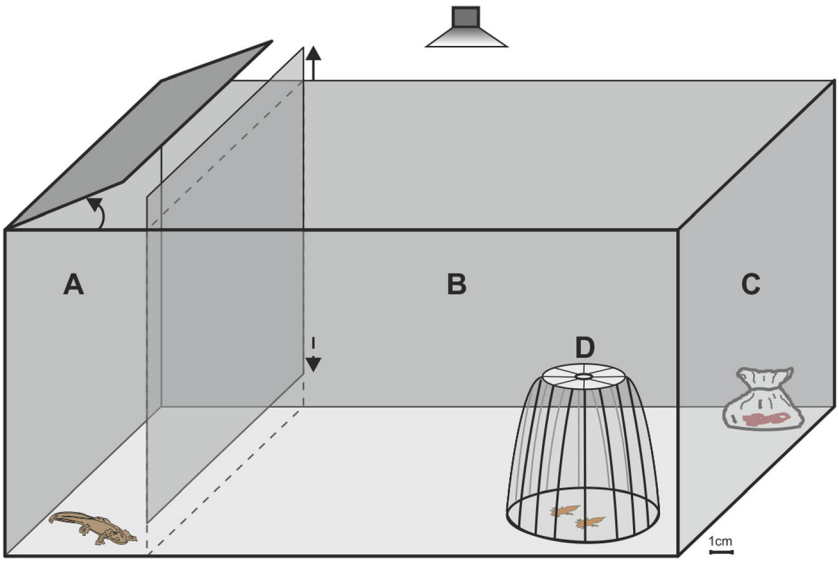


Fig. 1 The experimental setup: A refuge, B arena, C mesh bag with live *Tubifex*, and D cage with live dragonfly larvae. The size of organisms is increased for a better readability of the figure

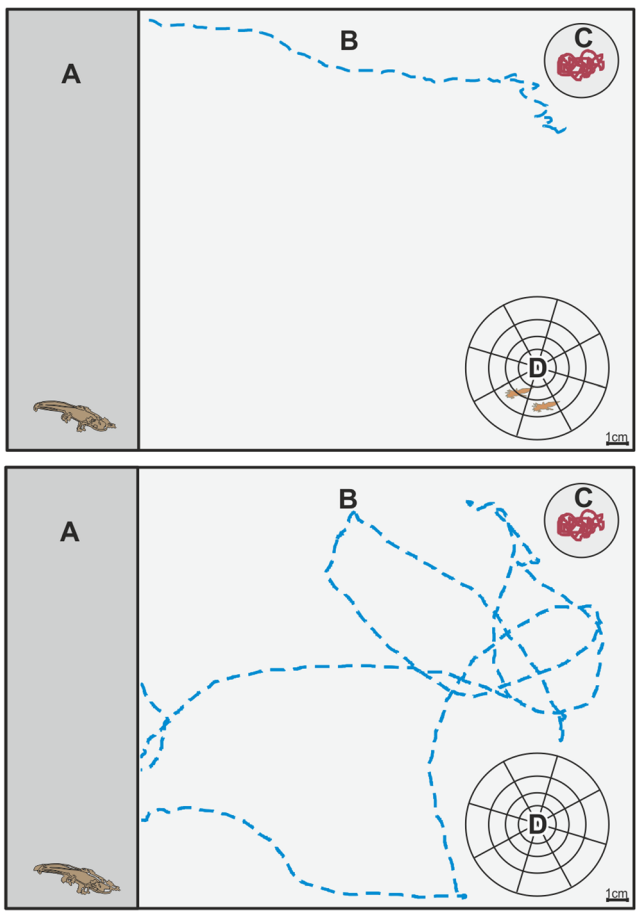


Fig. 2 Examples of tracks of movement during 15-min trials in a fire salamander larva, obtained from video-tracking software (Ethovision). Upper image—the larva was tested in the presence of an experimental stimulus, i.e., caged live dragonfly larvae, and lower image—the larva was tested in the absence of an experimental stimulus; A refuge, B arena, C mesh bag with live *Tubifex*, and D cage for predators

the cage and food bag in the left or right corner of the arena was randomized, to prevent cognition bias. The antipredator response of each fire salamander larva was tested three times in each risk scenario; we repeated the trials every third day, just before feeding, which allowed us to obtain a similar satiation level in salamander larvae among trials. We started with the no risk scenario trial and then alternated between risk and no risk scenarios. The order of individual larvae tested in each trial was randomized.

During each behavioral observation, a larva was placed in the refuge and allowed to acclimate for 5 min; then, the sliding door separating the arena from the refuge was lifted and the larva was allowed to move freely throughout the container (i.e., refuge and arena) during a 15-min time period. To prevent disturbing the focal salamander larva during recording, the experimental setup was placed behind a wall. Behavioral observations were recorded (25 images/s) from above, in the dark (average light intensity 0.01 lux), using an HD infrared camera (IP Hikvision DS-2CD2610F-1). Recording was done in the dark to mimic the natural conditions of foraging activity, since salamander larvae are largely nocturnal or crepuscular in feeding activities (Manenti et al. 2016). The water in the experimental containers was changed after each behavioral observation. At the end of a trial, the salamander larvae were returned to the rearing containers, and fed.

The video files were analyzed using Noldus Ethovision XT13 video-tracking software (Noldus Information Technology, Wageningen, the Netherlands). This software converted the images of the salamander larvae into pixels in each image and processed them across time to provide detailed quantitative data of the movement behavior (see Delcourt et al. 2013 for a review). The videos were processed at the original frame rate of 25 images per second (720 × 1280 pixels). The behavior of salamander larvae was quantified by

measuring (i) the refuge emergence, a binary variable scored as "1" if the larva left the refuge and "0" if the larva did not leave the refuge within a 15-min time period after acclimation; and (ii) distance moved, a continuous variable measured as the total distance moved in the arena from the time the larva left the refuge during the 15-min time period. As both the emergence from the refuge and the distance moved were captured automatically with the same settings by the behavioral software, there were no observer biases. After the behavioral observations, larvae were photographed on millimeter paper which was glued on the bottom of the rearing containers. Snout-vent length to the nearest 0.01 mm was measured for each salamander larva from the digital photographs using the software ImageJ v. 1.50i (Schneider et al. 2012). Measurements in ImageJ were conducted by a single person (IN).

Data analysis

We fitted a generalized linear mixed model (GLMM) with refuge emergence as the dependent variable, and a linear mixed-effects model (LMM) with the total distance moved as the dependent variable, respectively. The distance moved was log transformed. In the models, we included the risk scenario, food and light conditions, and population as fixed effects, and the identity of the larva as a random factor. The manipulation of both food and light prior to the behavioral trials resulted in differences in the size of salamander larvae (see Table S1 and 2 in the Supplementary Material), which could potentially influence their antipredator responses. Thus, we included in the models the SVL of salamander larvae after 2 months of exposure to different food and light conditions (hereafter SVL final) and population origin as covariates in the models. However, because neither population nor SVL final had significant effects and did not affect the statistical significance of other variables (see Table S3 in the Supplementary Material), we removed them from the subsequent analyses. We assumed a binomial error distribution and logit-link function for the GLMM, and a Gaussian distribution and identity link function for the LMM. As five larvae metamorphosed (two larvae in 0-h light, and high food treatment, two larvae in 8-h light and high food treatment, and a larva in the 8-h light, and low food treatment) before carrying out the third trials in both risk scenarios, the study of emergence from the refuge was based on 566 behavioral observations instead of 576 (96 larvae * three trials * two risk scenarios). The models were fitted using the glmer (GLMM) and lmer (LMM) functions in R (R Core Team 2020). In both cases, predictions and standard errors were generated with the bootMer function in the lme4 R package (Bates et al. 2015). To assess the significance of model predictors, we used Wald and likelihood-ratio chi-square tests (type II analysis of deviance) in R package "car" (Fox and Weisberg 2011). To examine differences among the predictors' levels, we used least square means (LS-means) for multiple comparisons and calculated the P values adjusted using the Tukey method.

To minimize observer bias, blinded methods were used when all behavioral data were recorded and analyzed.

Results

From a total of 566 behavioral observations, salamander larvae emerged from the refuge in total 316 times: 71 times in the high food 0-h light treatment, 65 times in the high food 8-h light treatment, 78 times in the low food 0-h light treatment, and 102 times in the low food 8-h light treatment.

The GLMM revealed that both risk scenario and food level had a significant effect on the probability that salamander larvae emerged from the refuge ($P < 0.01$ for both, Table 1). When exposed to the presence of dragonfly larvae (i.e., risk scenario), salamander larvae were significantly less likely to leave the refuge than in their absence (i.e., no risk scenario) (odds ratio = 0.581 ± 0.105, z ratio = − 3.008, $P < 0.01$, Fig. 3a). Salamander larvae at high food level had a significantly lower probability to emerge from the refuge than those at low food level (odds ratio = 0.512 ± 0.109, z ratio = − 3.147, P = 0.01, Fig. 3b). The main effect of light on the salamander larvae probability to emerge from the refuge was not significant ($P = 0.146$, Table 1).

The GLMM also revealed a significant interaction effect between food level and light conditions on the probability that salamander larvae emerged from the refuge ($P < 0.05$, Table 1). LS-means test showed that salamander larvae at low food level maintained in 8-h light conditions were significantly more likely to emerge from the refuge compared to those at high food level maintained both in 8-h light (odds ratio = 0.308 ± 0.095, z ratio = − 3.821, $P < 0.001$, Fig. 3c) and 0-h light conditions (odds ratio = 0.370 ± 0.114, z ratio = −3.240, $P < 0.01$, Fig. 3c). Salamander larvae at low food level maintained in 8-h light conditions showed significantly

Table 1 Results of the mixed models assessing the effects of risk scenario (presence versus absence of stimuli from caged larval dragonflies), food level (low versus high), and light conditions (0-h light versus 8-h light) on the refuge emergence and distance moved by fire salamander larvae

	χ^2	df	P
Probability of refuge emergence			
Risk scenario	9.046	1	0.003
Food	9.148	1	0.002
Light	2.113	1	0.146
Food:light	5.742	1	0.017
Log (total distance moved)			
Risk scenario	1.939	1	0.164
Food	2.106	1	0.147
Light	2.662	1	0.103
Food:light	1.716	1	0.190

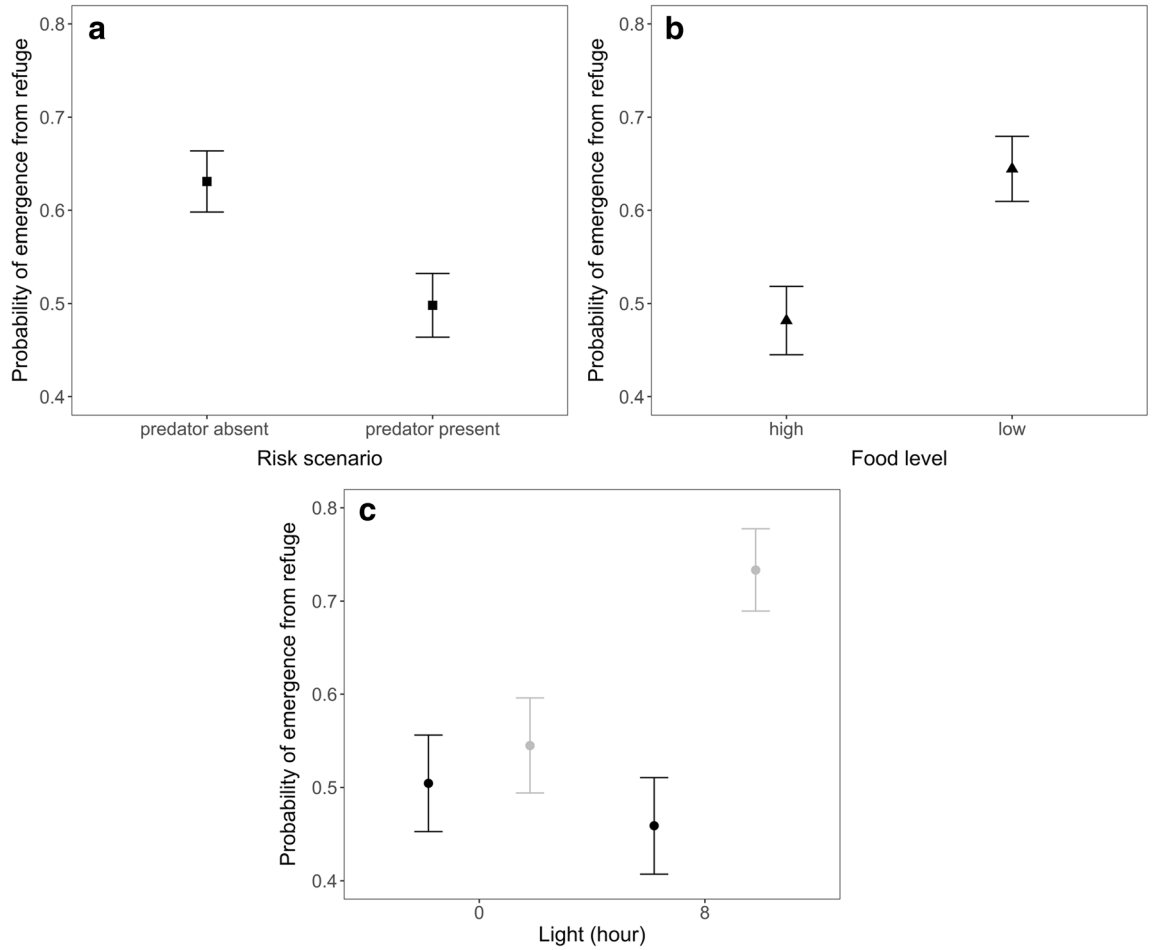


Fig. 3 The probability of refuge emergence in relation to risk scenario (presence versus absence of stimulus from caged dragonfly larvae), food level (low versus high), and light conditions (0-h light versus 8-h light) in the fire salamander larvae: a main effect of the risk scenario, b main effect of the food level, and c the two-way interaction effect between food level and light conditions (see Table 1 and text for statistical tests). In a and b, squares and triangles indicate the mean values of the probability to emerge from the refuge and bars represent standard errors (SE). In c, grey dots and lines represent the mean value and SE of refuge emergence of salamander larvae in the low food level, and black dots and lines those in the high food level, respectively

lower refuge emergence than larvae in the same food treatment but maintained in 0-h light conditions (odds ratio = 0.435 ± 0.132, z ratio = −2.734, $P = 0.05$, Fig. 3c).

The LMM revealed that neither the risk scenario, food level, nor light conditions had a significant effect on the distance moved by salamander larvae which emerged from the refuge ($P = 0.164$, $P = 0.147$, and $P = 0.103$, respectively, Table 1). The interaction effect between food level and light conditions on the distance moved by salamander larvae was also not significant ($P = 0.190$, Table 1).

Discussion

Food availability and light conditions are known to play a major role in the behavioral responses of ectotherms to predators, including those of amphibians (Lienart et al. 2014). However, whether the interaction of these environmental factors affects the antipredator behavior of amphibians remains poorly understood. The results of our study showed that food level and light conditions interact to shape the antipredator behavior of *S. salamandra*. Fire salamander larvae maintained for 2 months on low food levels and in 8-h light conditions took more risk (i.e., displayed significantly higher probability to leave the refuge) when exposed to larval dragonflies, compared to salamander larvae maintained in high food level and other light conditions. These results indicate that fire salamander larvae had the ability to modulate their behavior depending on immediate predation risk and past variation of environmental conditions. While Oswald et al. (2020) showed that risk-taking behavior of salamander larvae depends on the predation risk in the natal habitat, the results presented in this study show that risk-taking behavior is complex and depends on multiple environmental conditions. More generally, while it is well understood that the growth/predation risk trade-off is determined by an interaction

between food level and risk, our results indicate that the interactions with environmental factors may modulate the trade-off.

Our findings contribute to a growing body of literature on antipredator behavior in amphibians. Antipredator responses such as shelter-seeking behavior, an increased refuge use, spatial avoidance, and reduced activity have been all reported for amphibian species exposed to a predation risk (Holomuzki 1986; Semlitsch 1987; Sih et al. 1988, 1992; Lawler 1989; Alford 1999; Kats and Dill 1998; Van Buskirk and Schmidt 2000; Teplitsky et al. 2003; Winandy et al. 2016). In this study, we provided evidence that fire salamander larvae responded to a risky situation by significantly reducing the emergence from refuges. However, salamander larvae did not display a significant decrease of movement in the presence of a predation risk. The availability and use of a refuge has been associated with positive effects for the prey. For instance, the presence of a refuge contributes to a reduction of prey-predator encounter rates by increasing the habitat complexity or impairing visual abilities and movements of the predator (Sredl and Collins 1992; Babbitt and Tanner 1997). The refuge use implies a reduction of the time when prey is exposed to predators (Sih et al. 1988; Lawler 1989), which in turn increases prey survival. However, negative effects associated with prey refuge use have also been documented, such as a reduction of foraging opportunities with possible negative impacts on prey state (Sih 1987). Thus, our results suggest that salamander larvae evolved flexible strategies to use the refuge, in order to minimize the negative effects associated with the periods of time spent in the refuge (Martín and López 1999). This means that after adopting a less secure but needed response in a risky situation (i.e., emergence from the refuge), salamander larvae behaved in a similar way (i.e., moved similar distances) regardless of predation risk.

Food level is an important intrinsic determinant of activity levels in animals (McNamara and Houston 1986). In the present study, food level significantly affected the decision of fire salamander larvae to emerge from the refuge both as main factor and in interaction with light conditions. Poorly fed salamander larvae maintained in day/night cycle were significantly more likely to emerge from the refuge compared to those maintained in other food levels and light conditions. The foraging models of McNamara and Houston (1986) and Mangel and Clark (1986) predict that the decision of actions taken by an animal depends on its energetic requirements. Although many factors induce changes in the energetic requirements, it has been suggested that food availability and light conditions are key factors. Thus, the expected increase of energetic requirements in salamander larvae maintained for 2 months under low food and a day/night cycle requires them to forage more often and thus, to leave the refuge to achieve food intake.

As this study highlights, the risk-taking behavior is determined by environmental conditions. We found that predation risk, food level, and light conditions interactively determined the refuge-emergence probability of fire salamander larvae. However, light conditions alone did not significantly affect the risk-taking behavior of salamander larvae. These results emphasize the importance of understanding how the interactive effects of environmental conditions determine behavioral decisions. Moreover, whereas most experiments focused on the effect of short-term changes in the environment, our study mimicked the natural long-term exposure to an environment which differed in two crucial environmental parameters (i.e., the level of food resources and the regular interval of daylight and darkness). Short-term changes in environmental parameters play an important role in predator-prey dynamics, with implications for population dynamics (Lienart et al. 2014). However, understanding how longer-term environmental changes mediate behavior will certainly improve our ability to disentangle the contribution of behavioral plasticity to species persistence under environmental change. We therefore stress the need to consider multiple environmental effects and their interactions when studying behavioral variation in animal populations and the importance of assessing the long-term changes in the environment on behavioral responses.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-021-02966-w.

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Authors' contributions RIB, FS, and MD conceived the idea of the study. RIB, FS, and BRS analyzed the data. RIB and MD wrote the manuscript with input from FS and BRS. MD supervised the work. RP and IN contributed to the design and carrying out the behavioral experiments.

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Data availability The dataset analyzed during the current study is available in the KNB repository: https://knb.ecoinformatics.org/view/urn%3Auuid%3A7d01db76-0c1a-429c-a442-a8788e85f419.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval Because this study was only observational and not experimental with regards of laboratory research on animals, it did not need specific ethical approval. The collection, captive maintenance, and observation procedure of the fire salamander larvae complied with all relevant guidelines, notably the Directive 2010/63/UE of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Romanian Speleological Heritage Commission issued environmental permit no 78/10.02.2016. Both 8-h and 0-h light experimental conditions used to rear the fire salamander larvae mimicked natural situations, as this species is naturally present in these two situations in the wild as they can be present in caves (Manenti et al. 2011). No larva died during the experiment.

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