

# Landscape of fear in freshwater ecotones: How predation risk and light conditions affect mesopredator activity and foraging in springs

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## Abstract

1. Springs are environments that can provide general insights into factors favouring diversity in ecotones, but they are often neglected in freshwater studies. One of the challenging processes acting in ecotones is the landscape of fear (LOF), the space–time variation of perceived predation risk. Spring exploitation often involves species that are mesopredators in surface fresh water and that can become apex predators in ground water, as in the case of the fire salamander larvae (*Salamandra salamandra*). Here, we aim to determine whether the activity and foraging patterns of the fire salamander in springs are affected by LOF.
2. We surveyed the night- and daytime abundance of fire salamander larvae in 15 springs to assess predator occurrence. We also reared 48 salamander larvae with and without non-lethal exposure to predators within tanks simulating groundwater or surface freshwater light features. Before and after a month of rearing, we tested larva efficiency in catching prey when exposed to predator chemical cues, both in light and dark conditions.
3. In the field, the number of active fire salamander larvae was significantly higher during the night. At night, the number of active larvae across the transition area between ground water and surface water was higher in plots closer to the surface.
4. Testing and rearing conditions significantly affected larva behaviour, and prey capture was significantly more effective in light conditions. It was less successful in larvae reared with predator chemical cues and in the presence of predators. Moreover, larvae reared with predators under light conditions were slower than those raised with predators in dark conditions.
5. Our results show that LOF can interact with extant environmental features and constitute a significant behavioural pressure for mesopredator species living in freshwater ecotones.

## KEYWORDS

amphibians, dragonfly, interface, predator, source

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## 1 | INTRODUCTION

Surface fresh waters are characterised by complex interactions between multiple abiotic and biotic factors that shape the communities inhabiting them through multiple functional, behavioural, genetic, plastic and adaptive processes (Colgan & Ponder, 1994; Dodds et al., 2019; Ficetola et al., 2021; Moss et al., 2005; Silknetter et al., 2020). Research on ground waters has increased in recent years, with growing recognition of their key role. Although groundwater studies pose several difficulties in terms of accessibility and logistics, they can be attractive from a research perspective because they are simpler environments compared to their surface counterparts. Biotic features are constrained by relatively few key factors such as light absence, lower oxygen content and limited nutrient availability (Culver & Pipan, 2014; Romero, 2020). Consequently, the trophic web is reduced and cave-dwelling animals face safer and more stable conditions than animals in surface waters (Manenti, Melotto, et al., 2020). Much less studied, or seldom considered except as an appendix to the surface or subterranean compartments, are springs. Springs are typical ecotonal habitats where subterranean and surface pressures interact and organisms interplay dynamically in shaping communities (Alfaro & Wallace, 1994; Cantonati et al., 2006). The study of springs can be particularly promising for ecotone research. They provide opportunities to disentangle the role played by the main ecological processes acting at the boundaries and shaping the transition between two distinct environments. They also offer general insights applicable to other environmental continuums occurring in different systems.

In springs, the interaction between the environmental continuums acting in surface and in ground water can produce contrasting pressures on organisms. Indeed, while groundwater-dwelling animals can be attracted to an ecotone spring area because of its high food availability, conditions in these environments also can be detrimental, as organisms can suffer increased predation pressure from surface predators and can be affected by UV radiation during daytime (Manenti & Piazza, 2021). However, cave animals have adaptations to cope with darkness, and this may foster shifts in diel activity, favouring night exploitation of ecotones. Thus, if spring exploitation provides advantages in terms of energy income, it is possible that groundwater-dwelling animals develop diel activity changes in antipredator responses or morphological adaptations (pigmentation) to contrast surface constraints. In the long term, these processes also may foster the colonisation of the surrounding surface environments, as suggested by some lineages of crustacean amphipods that probably have colonised surface environments (Copilas-Ciocianu et al., 2017). Conversely, for surface animals, springs can be attractive, since they offer durable hydroperiods, greater microclimatic stability and lower predation pressures than downstream sites. At the same time, however, springs can face scarcity of trophic resources. Nevertheless, if spring exploitation provides advantageous conditions to surface animals and sufficient resources are available, local adaptation and/or plastic shifts in traits favouring the exploitation of the groundwater side of springs may occur. This process has

been demonstrated in several surface species colonising ground water (Dreiss et al., 2009; Issartel et al., 2010; Limongi et al., 2015; Manenti, Siesa, & Ficetola, 2013; Salin et al., 2010). Often, groundwater colonisation involves species that are mesopredators in surface freshwater and spring environments, as in the case of the larval stage of the fire salamander (*Salamandra salamandra*). Fire salamander larvae are common in European springs and can strongly affect spring trophic webs (Barzaghi et al., 2017). When occurring in surface creeks and springs, salamander larvae are typical mesopredators, preyed upon by dragonfly larvae (Bancila et al., 2021; Barzaghi et al., 2020). Conversely, when in caves, salamander larvae generally occupy an apex position in the food chain, as their only predators are larger conspecifics (Manenti, Lunghi, et al., 2020; Melotto et al., 2019). At the same time, the occurrence of salamander larvae in springs seems to limit their exploitation by subterranean-dwelling invertebrate prey (Manenti & Pezzoli, 2019). Assessing factors limiting or enhancing patterns of activity and occurrence in spring habitats of key predator taxa, such as the fire salamander, can provide relevant insights into the pressures shaping diversity in ecotone communities. One of the main biological processes that is likely to shape activity and distribution in a variety of ecotones is the landscape of fear (LOF).

The LOF concept is a behavioural trait that can be used both at the individual and population levels (Bleicher, 2017; Gaynor et al., 2019; Matassa & Trussell, 2011). The LOF provides a space-time-supported measure of the way an animal or a population of animals "perceives" the surrounding environment based on the trade-off between exposure to predation risk and activity patterns within specific areas/habitats (Bleicher, 2017). The LOF can be affected by a large variety of biological, ecological and evolutionary variables (Bleicher, 2017; Gaynor et al., 2019). Prey must adopt strategies optimising the balance between predator avoidance and the time they can devote to key functions, such as foraging. Thus, a forager has to modulate its patterns of activity according to the perceived level of risk, selecting those spatio-temporal patches limiting risk exposure while foraging (Matassa & Trussell, 2011). Animals navigating heterogeneous landscapes experience different LOFs (Gaynor et al., 2019). The activity patterns of mesopredators can be strongly affected by risk exposure. For instance, top predator presence can dampen both the prey search and prey capture rates (Kishida et al., 2011). Thus, in habitats with abundant predators, such as surface environments, mesopredators are expected to show different activity patterns than their counterparts that exploit safer habitats, such as ground waters. Both the diversity of the predator community and the features influencing predator activity and behaviour play major roles in affecting risk exposure and shaping LOF (Gaynor et al., 2019). As an example, predator diel activity patterns may strongly change the features of LOF (Bleicher et al., 2019; Laundre, 2010) with consequent repercussions on prey activity itself. This happens, for example, in streams where fire salamander larvae occur: some of their prey, such as mayfly larvae of the family Baetidae, are drifted more by water flow during the night, when salamanders seem more active, than during the day (Oberriesser & Waringer, 2011).

In this paper, we wanted to investigate the activity patterns of fire salamander larvae in spring habitats, and how LOF and ecotonal conditions can affect their variation. To this end, we coupled field surveys with a cross-environment experiment and surveyed salamander larvae abundance in spring habitats at different distances from the boundary between surface and ground water. We surveyed habitats where predators occurred and where they were absent. We also reared salamander larvae at different LOF levels in tanks simulating groundwater and surface light occurrence. We hypothesise that LOF in springs reduces mesopredator activity, while we predict that stronger LOF in experimental conditions limits effectiveness in reaching prey. This effect is further enhanced by light, the variation typically characterising surface environments. Accordingly, we predict that salamander larvae show stronger diel activity variations in the surface side of springs where they are exposed to predation risk than in ground water, where they are the apex predator.

## 2 | MATERIALS AND METHODS

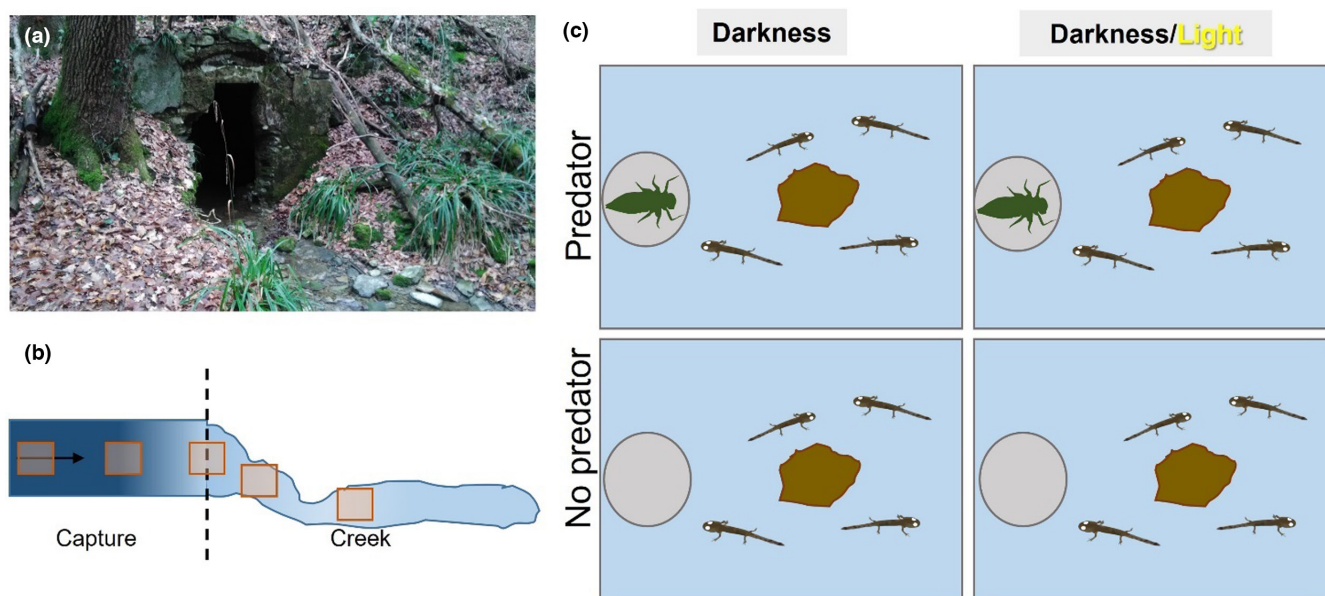
### 2.1 | Field activity

From March to May 2021, we monitored 15 springs between the Lecco and Como districts (Lombardy, northern Italy, approximately 45.8N, 9.4E). Each spring was divided into subsections, hereafter plots (1–4 plots for every spring), randomly placed across the boundary between ground water and the surface from 2 m on the surface side to 7 m underground; the average plot length was 126 cm and average plot width 90 cm (Figure 1). A total of 25 plots were surveyed

twice at night and twice during the daytime. The same observer (SL) performed all of the surveys. During each survey, we recorded for each plot the number of active fire salamander larvae (i.e., the number of larvae detected in 10-min visual surveys). At the end of each night survey, and at least 7 days before the successive survey, we also performed a dip-netting sampling in each plot by moving the substrate for 5 min and using a thin-mesh dip net to collect all of the salamander larvae and all of their potential aquatic predators occurring in the plot.

### 2.2 | Experimental setting

In April 2021, we collected 48 newborn fire salamander larvae from two different rheocrene springs (24 larvae from each site) of the karst locality of Alpe del Vicerè (Como district, NW Italy). Larvae were collected within 10 m downstream of each spring site. In these spring sites, only the surface is accessible to both salamanders and humans. Larvae were transferred to the subterranean biology laboratory “E. Pezzoli” (Galbiate, NW Italy) and then equally divided into 12 microcosms (four larvae per microcosm). The microcosms were 40×30×22 cm tanks, with 8 cm of water depth sharing the same features: a 5-cm stone as shelter on the right side, two feeding bowls (white plastic bowls 6 cm in diameter) and a pierced transparent bottle (Ø = 10 cm). During the rearing period, larvae were exposed to two different conditions: control (no predator) and risk (non-lethal predator presence), generating different LOF conditions. One dragonfly larva (*Cordulegaster boltonii*), acting as the top predator in the system, was confined in the transparent perforated bottle in risk



**FIGURE 1** Study setting. (a) Example of unmanipulated field sampling site formed by a spring with its downstream creek. (b) Example of sampling schema of a spring; the arrow shows waterflow direction; dashed line shows the edge between ground water and surface water; squares exemplify sampling plots; number and position of plots varied with spring features, in some sites only groundwater, edge or surface plots have been sampled. (c) Microcosms setting for experimental rearing of fire salamanders (in black); brown form shows shelter; grey circle shows dragonfly larva (in green) cage.

conditions, whereas the bottle was empty in the controls (Figure 1). Dragonfly larvae belonged to the same spring site, with sizes varying from 3.1 to 3.7 cm. Salamander larvae were free to wander throughout the microcosm. Microcosms were characterised by two distinct light conditions. Half of the microcosms were set in constant darkness, whereas half were set with a varying photoperiod of 12 hr (from 07:00 to 19:00 hr), facilitated by a NICREW 3W 28 cm white LED light for aquariums, which provided an illuminance of 1800 lux. All tanks were aerated with a Zacro air pump and had the same water temperature (12°C). During rearing, we fed fire salamander larvae ad libitum every 2 days with live *Chironomus* sp. larvae. Chironomids were placed in the feeding bowls to limit their dispersal. Chironomids also were provided ad libitum to *C. boltonii* larvae for a total of 10–12 prey per week. Rearing lasted 30 days. Moreover, both at the beginning and at the end of the rearing period, each larva was photographed laterally to allow individual identification (Eitam & Blaustein, 2002; Romeo et al., 2015). This identification method enables the accurate identification of salamander larvae in field conditions, and it was particularly effective owing to the limited number of larvae per tank and laboratory conditions. At the end of the rearing period, we assessed the number of surviving larvae in each tank.

### 2.3 | Behavioural tests

Behavioural tests were performed in two separate sessions, at collection (after 3 days of housing) and at the end of the rearing period (after 30 days). The tests consisted of measuring the efficiency of salamander larvae in reaching prey in the presence or absence of risk cues. Before performing the tests, we kept the salamander larvae without food for three days so that they all would have the same level of hunger. This absence of feeding does not affect salamander growth and naturally occurs in the wild (Cogliati et al., 2022). During behavioural tests, larvae were exposed to predator chemical cues or water control, both in total darkness and in light (700 lux) conditions. Predator cues were obtained by maintaining six wild-caught *C. boltonii* larvae for 24 h in 1.5 L of dechlorinated tap water. The water was aliquoted (1 ml) and immediately stored at -20°C until used for the tests, following a standard procedure (Epp & Gabor, 2008). As control cues, we used 1 ml of dechlorinated tap water. Each larva was tested twice for each possible combination of cue exposure and light condition (with or without predator cues both in light and total darkness, in two replicates per test, i.e.,  $N = 8$  tests per larva on each session). The identity of the larvae to be tested in each trial, the light conditions of the test and the chemical cue treatment order were randomly selected until each larva was tested twice per each test combination. Before each test, larvae were placed for 3 min in tanks filled with tap water. During the behavioural tests, each larva was individually placed in a 13.5 × 18.3 cm plastic container filled with 5 cm water and was allowed to acclimatise for 3 min. At the beginning of the test, one live chironomid prey larva was placed in the opposite side from where the salamander larva was (at a distance of ~16 cm) and 1 ml of water with the test cues (predator cues or control water)

was cautiously placed with a pipette in the middle of the arena. We then recorded whether each larva caught the prey. Trials lasted until the larvae bit the prey or for a maximum of 7 min if the chironomid was not reached. In total darkness, we used an IR night visor to observe salamander behaviour.

### 2.4 | Data analysis

In order to assess if diel activity of fire salamander larvae in the field was affected by LOF and ecotone level, we used a generalised linear mixed effect model (GLMM). In the analysis, we considered only plots belonging to spring sites in which the occurrence of salamander larvae was assessed at least once by dip-netting or visual surveys. The number of visually recorded larvae in each field survey (pooled across the plots) was the dependent variable. As fixed factors, we considered the period of the day (night or day), distance from the boundary, and top-predator status (if salamander larvae were or not the top predators at the whole spring site). We also preliminarily tested all possible two-way interactions between the fixed factors included in the model. In the final model, we included significant interactions only (Equation 1). We used a negative binomial error distribution to take overdispersion into account. Plot and spring identity were included as random factors to take into account the non-independence of observations (Pinheiro & Bates, 2000).

#### Number of active fire salamander larvae

$$\begin{aligned} &\sim \text{Period of the day (day/night)} \\ &+ \text{Fire salamander larvae as top predators (yes/no)} \\ &+ \text{Period of the day (day/night)} \\ &*\text{Distance from the boundary (positive = surface; negative = ground water)} \end{aligned} \quad (1)$$

To assess how rearing conditions interacted with light treatments and predator cues in affecting salamander predation effectiveness, we built a GLMM. The dependent variable was the success/failure of prey biting by salamander larvae. As fixed factors, we considered light rearing conditions (darkness, light/darkness), predator rearing conditions (with or without predators), light test treatment (light or darkness) and predator cue treatment during the test (predator cues or control). We also tested all the possible two-way interactions between all the fixed factors and included only those that were significant in the final model (Equation 2). The period of rearing (beginning or end of the rearing) was included in the model as a covariate. Larva identity, test replicate (first or second) and tank of rearing were random factors. We also tested overdispersion issues by preliminarily building the same model without mixed-effect error (generalised linear model, GLM) and using quasibinomial distribution.

$$\begin{aligned} &\text{Successful prey reaching (Y/N)} \sim \text{Predator test} \\ &+ \text{Light test} * \text{Light rearing} + \text{Predator rearing} \\ &+ \text{Rearing period} \end{aligned} \quad (2)$$

Finally, we also tested whether rearing conditions affected larval survival using a GLM with binomial error distribution. We considered as the dependent variable a two-column matrix composed of the number of survivors and the number of the dead (Equation 3), using counts across microcosm replicates within each treatment at the end of the rearing period.

$$N \text{ of survivors, } I(N \text{ of dead larvae}) \sim \text{Light rearing condition} + \text{Predator rearing condition} \quad (3)$$

We assessed the significance of the fixed factors of all of the models using Wald  $\chi^2$  tests (Bolker et al., 2008), and we checked model assumptions by verifying the absence of multicollinearity issues through VIF calculation (Zuur et al., 2010). All statistical analyses were performed in R 4.1.1 using *lmerTest*, *MuMIn* and *glmmTMB* packages.

### 3 | RESULTS

#### 3.1 | Field assessment

In the field, we detected fire salamander larvae in 11 springs; larvae were detected at least once in all plots except one. The maximum number of larvae counted in each survey was 70. Fire salamander larvae were the apex predators in six springs, while in the remaining five sites, odonatan larvae (genus *Cordulegaster* or *Aeshna*) occurred in at least one plot. Additionally, in one spring, we recorded the occurrence of adult, white-clawed freshwater crayfish (*Austropotamobius pallipes*), which also can feed upon salamander larvae. The number of active salamander larvae was significantly higher during night surveys ( $\chi^2_{1,50} = 9.2$ ;  $p < 0.01$ ). Moreover, during the night, the number of active larvae was higher in the surface end of plots than in the groundwater one ( $\chi^2_{1,50} = 8.93$ ;  $p < 0.01$ ; Figure 2). Predator status in

the spring site did not affect the number of active larvae observed ( $\chi^2_{1,50} = 4.67$ ;  $p = 0.49$ ).

#### 3.2 | Experimental assessment

At collection, the average total length of larvae placed in experimental conditions was 32.4 mm (range 26.7 to 33.7 mm); at the end of the 30 days of rearing, larvae reached an average total length of 38.04 mm (range 33.9 to 42.6 mm). The total survival rate was 60.4%. In LOF rearing conditions, nine of 24 (37.5%) larvae survived, whereas without LOF, 20 of 24 (83.3%) larvae survived. Larvae that did not experience LOF during rearing had a significantly greater rate of survival ( $\chi^2_{1,11} = 8.49$ ,  $p < 0.01$ ); light conditions did not significantly affect survival ( $\chi^2_{1,11} = 1.05$ ,  $p = 0.30$ ).

After 1 month of rearing, larvae were significantly more efficient at catching prey (Table 1). Test treatments significantly affected larvae behaviour: larvae captured prey significantly more in light and without predator chemical cues (Table 1; Figure 3). LOF (rearing with predators) significantly affected performance: larvae reared in LOF conditions were less efficient at catching prey (Table 1; Figure 3). Conversely, light conditions experienced during rearing did not significantly affect prey caught during the tests. We also detected a significant interaction between light conditions during rearing and at the test: during tests without light, larvae reared in total darkness showed greater ability in catching prey (Table 1; Figure 4).

### 4 | DISCUSSION

Our results reveal that fire salamander larvae show marked nocturnal habits in spring habitats; particularly intriguing is the fact that differences in diel activity occurred between surface and underground plots of the springs, with a higher number of

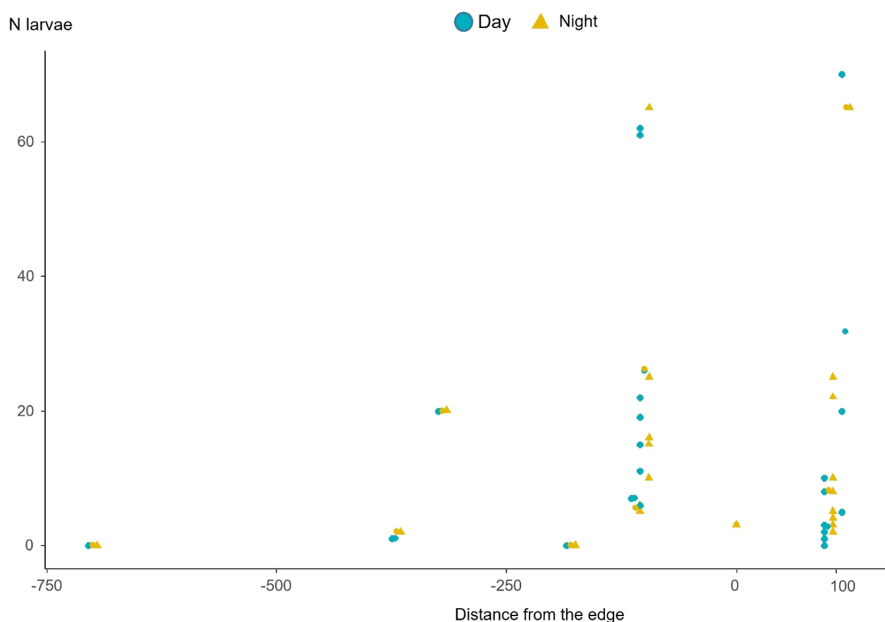


FIGURE 2 Interaction between day/night period and distance from the edge in affecting fire salamander larvae number in the sampled plots. Points represent individual counts of larvae during the different surveys.

salamanders active during the night in external plots than in internal ones. These results are in line with the observation that, in surface habitats, fire salamanders are often more active at night (Oberrisser & Waringer, 2011; Sanchez et al., 2019). Moreover, we obtained substantial evidence that the activity of mesopredators in underground habitats (i.e., where exposure to other predators is lower) can be generally more constant/higher than in surface habitats, where the occurrence of predators and light increases predation risk.

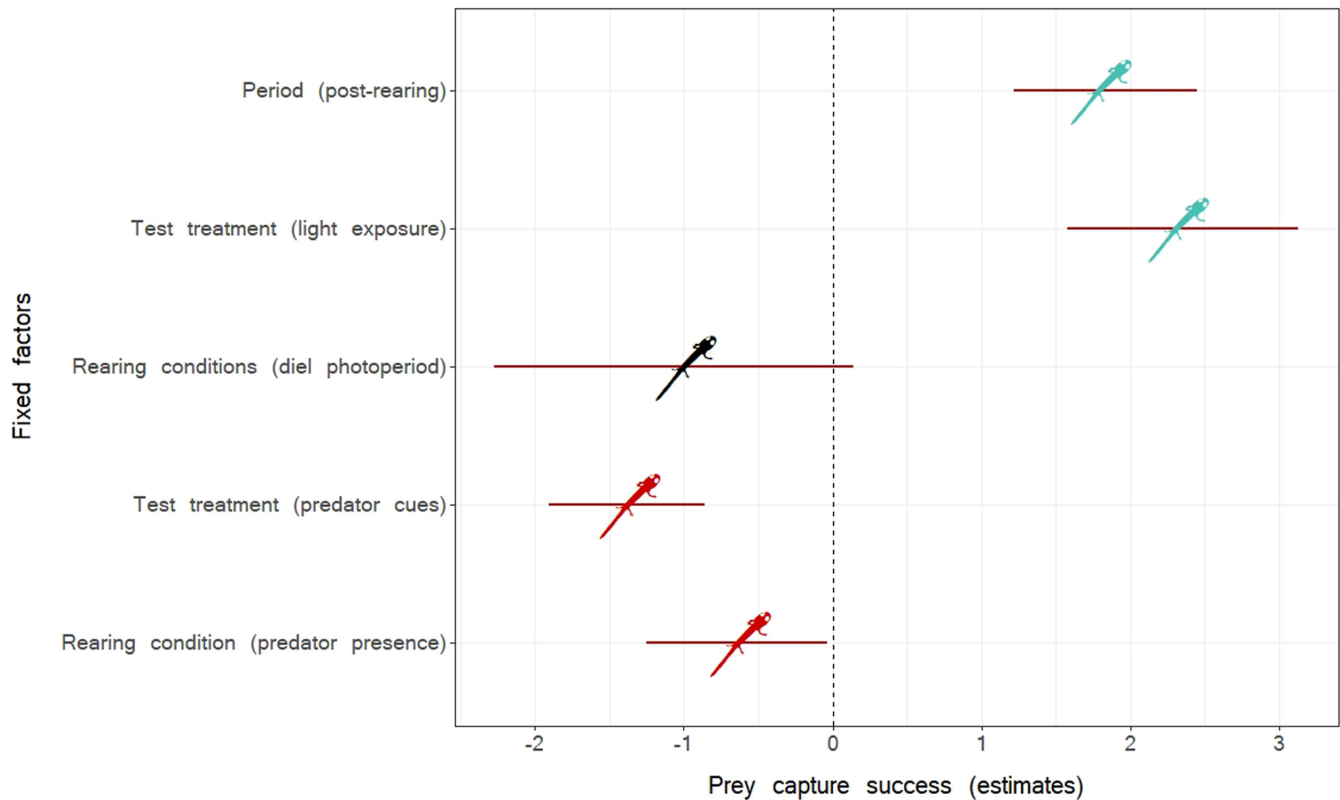
**TABLE 1** Results of the generalised linear mixed model on the effect of rearing and test conditions affecting prey-catching success of the fire salamander larvae (model described by Equation 2 in Material and Methods section).

	Estimate	$\chi^2$	<i>p</i>
Period of rearing	1.80	33.78	<0.001
Light test conditions	2.32	66.45	<0.001
Light rearing conditions	-0.99	0.04	0.84
LOF rearing conditions	-0.62	4.27	0.03
Cues test conditions	-1.37	26.77	<0.001
Light test conditions: light rearing conditions	1.29	3.96	0.04

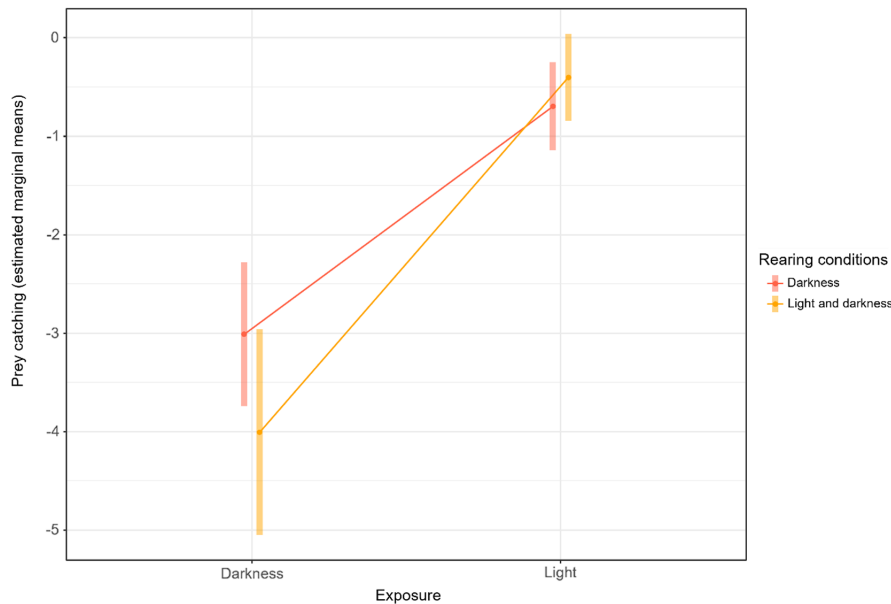
Note: All possible interactions between the fixed factors have been preliminarily tested, but only the significant ones were included in the final model.

However, the strength of the link between fire salamanders' diel activity and their perceived LOF is questionable. Observed diel activity patterns may simply reflect an intrinsic circadian rhythm typical of numerous epigeal species (Hoenen & Gnaspini, 1999; Merritt & Clarke, 2011), including fire salamanders (Himstedt, 1971), or may be affected by other extrinsic factors, such as predation risk (Winandy et al., 2016). If variation of diel activity was caused only by intrinsic factors, we would not expect differences along the spring ecotone, whereas our surveys revealed that during night the number of active larvae increased at the surface end of the spring only. At the same time, in the unmanipulated field conditions, the hypothesised connection between fire salamander activity and predator occurrence was not recorded, nor did its interaction with the period of the day affect activity patterns. This finding is in line with previous field studies performed in the cave and stream breeding sites of this species, which did not record differences in the frequency of active larvae between the two habitats when taking into account the effect of light and predator abundance (Manenti et al., 2016). Conversely, under controlled experimental conditions, predation effectiveness was significantly affected both by the LOF experienced during larval development and with the exposure to predator chemical cues during the behavioural test.

Our study confirms that visual perception is important to allow prey detection by fire salamander larvae. Salamander larvae are known to have dichromatic colour vision (Tempel & Himstedt, 1979)



**FIGURE 3** Factors affecting prey-catching success during behavioural test by fire salamander larvae. Red bars represent 95% confidence intervals, whereas salamander icons are the difference in the estimated mean. Green icons indicate significant positive effects, red icons underline significant negative effects, and black icons with confidence intervals overlapping zero are non-significant effects.



**FIGURE 4** Interaction between light conditions during rearing and at the test in affecting successful prey capture by fire salamander larvae. Points represent estimated means, vertical bars are 95% confidence intervals.

and to strongly rely on visual cues for other key fitness-related functions, such as escaping predators (Melotto, Ficetola, Pennati, et al., 2021). The success of prey capture was significantly higher under light than in dark conditions. Amphibian species that live in environmental contexts where visibility is not limited, such as clear, open freshwater habitats, are inclined to rely primarily on visual cues to detect prey (Lindquist & Bachmann, 1982). Changes in light conditions can affect the foraging behaviour of salamanders; in *Plethodon cinereus*, visual cues seem to be the primary mode of prey detection used during the daytime and linked to ambush foraging strategy, while during the night, this salamander can switch to a more active foraging behaviour that involves the use of chemical cues to detect prey in total darkness (Placyk Jr & Graves, 2001). A similar situation also has been described in fire salamander larvae that can switch from ambush mode to active foraging strategy depending on light conditions; however, larvae born in caves are better able to perform this switch than those born in streams and springs (Manenti, Denoël, & Ficetola, 2013), as were those used in this experiment. Nevertheless, variations in the effectiveness of visual cues during the day and during the night, together with associated activity patterns and predation risk, can strongly complicate the picture detailed from field observations.

Coupling field observations with experimental rearing in controlled conditions is fundamental to determining the role of processes shaping behavioural patterns (Peacor et al., 2022). However, environmental complexity can mask the importance of some processes, especially in a predator–prey context, in which multiple predators and prey can interact. Previous studies have shown that a combination of direct predatory activity, non-consumptive effects caused by predation attempts, and intense competition for common trophic resources can jointly affect the activity patterns and fitness of amphibians (Cabrera-Guzmán et al., 2017; Gomez-Mestre & Diaz-Paniagua, 2011). In our study, the complexity of field conditions also may be increased by the fact that LOF in

external plots in springs can be the result not only of aquatic predators, but also of terrestrial and semi-aquatic predators. Although often underestimated, predation by diurnal birds and mammals of amphibian larvae can be relevant (Gontijo et al., 2018; Hadad et al., 2022; Martins et al., 2021). For example, blackbirds and small opportunistic mammals could easily prey on salamander larvae, especially in confined environments such as small springs. The nocturnal habits of salamander larvae in spring habitats, especially on their surface side, also can help to avoid these predators. Further studies assessing the whole LOF occurring in springs, including birds and mammals would be useful. Higher nocturnal activity also could be related to the necessity of avoiding UV radiation during the daytime, especially UV-B radiation that can affect freshwater organisms (Alves et al., 2020; Cywinska et al., 2000). Such a strategy has often been described in invertebrates (Ciros-Perez et al., 2015; Rudh & Qvarnstrom, 2013). In springs, the occurrence of a more pronounced nocturnal activity has been recorded in stygobiont crustaceans of the genus *Niphargus* (Manenti & Barzaghi, 2020), which are totally depigmented and likely to be more sensitive to UV radiation exposure. However, fire salamander larvae possess melanophores and are thus provided with photoprotective compounds (Segev, 2009; Vlad et al., 2020). Consequently, the actual impact of UV radiation on these animals requires additional assessment.

In line with previous studies (Manenti et al., 2016; Melotto et al., 2019; Melotto, Ficetola, Alari, et al., 2021), the experienced LOF strongly affected mesopredator behaviour and performance, with both exposure to the predator during rearing and to its cues during testing negatively impacting prey-catching success (Figure 3). LOF effects apparently also acted on survival during the rearing period, as significantly more salamander larvae died in tanks with the predator. Even if dragonflies were confined and not able to catch the salamanders, it is possible that by protruding their mandibles, they caused some damage to salamanders swimming close by. In addition,

it is likely that LOF rearing conditions would have been more stressful, as already evidenced in invertebrates reared with high levels of predation risk (Hintz & Relyea, 2017). Furthermore, after a month of rearing, salamander larvae improved their effectiveness at catching prey. This is a well-known pattern already observed in this species (Melotto et al., 2019) and is probably linked both to the increased experience and increased swimming ability attained by salamanders through growth.

The strong effect of LOF observed in experimental conditions, together with the confirmation of the hypothesis that activity patterns vary with the distance from the boundary between surface and ground water, show that predator-prey interactions are important in allowing ecotone exploitation. Taken together, field and experimental results suggest that LOF can provide significant pressures for mesopredator species living in ecotones, shaping their activity and predatory strategy or predation success according to the extant ecotone conditions. Springs, being favourable environments for both different surface mesopredator species and surface and subterranean prey, can be excellent environments in which to assess the magnitude of predation-risk effects and how they can influence species abundance and community attributes. Moreover, springs, representing the boundary between two totally different environments, provide important systems for further investigation of the processes promoting diversity and adaptation of both predators and prey in ecotones.

#### AUTHOR CONTRIBUTIONS

Conceptualisation: RM, AM. Developing methods: MF, SL, MG, BB. Data analysis: RM, GFF. Preparation of figures and tables: AM. Conducting the research, data interpretation, writing: RM; GFF, AM, MF, SL, MG, BB.

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We are grateful to M. Villa of the Regional Park of Monte Barro. The rearing experiment was planned following ABS/ASAB guidelines for the ethical treatment of animals. The collection and maintenance of fire salamander larvae and their prey and predator invertebrate species was in accordance with Regional Law 10–31/03/2008 (Lombardy Region). The study design was approved by the regional ethical committee (permit no. 18303\_13.12.2019). Each individual of the different species was released at the collection site after the experiment.

#### CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

#### FUNDING STATEMENT

This research received no external funding.

#### ETHICS STATEMENT

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