

Foraging plasticity favours adaptation to new habitats in fire salamanders



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Predators often show strong plasticity of optimal foraging strategies. A major difference in foraging strategies occurs between sit-and-wait and active predators. Models predict that the efficiency of these strategies is affected by environmental conditions, active predators being favoured when prey are scarce and their detection difficult. The shift between the two strategies may occur through both phenotypic plasticity and local adaptations. Larvae of the fire salamander, *Salamandra salamandra*, are typically stream-dwelling sit-and-wait predators, but some populations breed in caves. We evaluated whether local adaptations or phenotypic plasticity determine shifts in foraging strategy between stream and cave populations. The foraging behaviour of salamander larvae was evaluated under all combinations of three test conditions during trials: light versus darkness, prey presence versus absence and food deprived versus fed; larvae originated from caves and streams and were reared in epigeous photoperiod or in darkness. Observations and video tracking showed that salamander larvae modified their behaviour in response to environmental conditions. In the darkness, larvae showed higher average velocity and moved longer distances. Movements were higher in food-deprived larvae and in the presence of prey compared to fed larvae and prey absent conditions. Furthermore, larvae from cave populations showed higher behavioural plasticity than stream larvae, and better exploited the available space in test environments. Variation in foraging behaviour was strong, and involved complex interactions between plasticity and local adaptations. Larvae from cave populations showed higher behavioural plasticity, supporting the hypothesis that this trait may be important for the exploitation of novel environments, such as caves.

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When populations colonize new environments, selection pressures may determine phenotypic divergence for multiple traits, including morphology, physiological tolerance and behaviour (Kawecki & Ebert 2004). Phenotypic divergence can occur through both local adaptations and phenotypic plasticity. First, divergent selection may favour genotypes determining locally adapted ecotypes. Second, one single genotype can determine multiple phenotypes (phenotypic plasticity), enhancing fitness under specific environmental conditions (Lande 2009; Torres-Dowdall et al. 2012). However, local adaptation and plasticity are not mutually exclusive and, under certain conditions, natural selection may favour individuals that are able to express strong reaction norms, showing the highest values of phenotypic plasticity. This can be particularly valuable when the colonized habitats are

heterogeneous, in the presence of gene flow between habitats, and during the early phases of the adaptation process (Crispo 2008). The interplay between local adaptation and plasticity is rarely assessed in natural populations, but may be detectable by ecotype-by-environment interactions, that is, when the slope of reaction norms differs between ecotypes (Crispo 2008; Torres-Dowdall et al. 2012).

Predators often show strong plasticity for the foraging strategies employed because optimal foraging can be influenced by multiple factors, such as physiological constraints, features of both the environment and prey, and risks associated with the predatory behaviours (Delclos & Rudolf 2011). A major difference in foraging strategies occurs between the sit-and-wait (also named 'ambush') predators, and the active (also named 'widely foraging') predators (Huey & Pianka 1981). Sit-and-wait predators usually remain hidden for more or less long periods waiting for prey to come close. In contrast, active predators widely explore their habitat (Scharf et al. 2006), performing wandering displacements that may increase the probability of encountering prey (Hodar et al. 2006). These two

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strategies are most effective under opposite environmental conditions. The sit-and-wait strategy requires the occurrence of moving prey and the possibility to detect it at long distances, while active predators are favoured when prey are encountered infrequently, when predators can move faster than their prey, and when predators' energy requirements are low in relation to the prey capture rate (Huey & Pianka 1981; Scharf et al. 2006). Sit-and-wait and active search are two extremes of the foraging mode spectrum, and predators can modify their strategies in response to the environmental context and prey identity (Perry 1999; Scharf et al. 2006).

Hypogeous (i.e. underground) environments have multiple features that differentiate them from epigeous habitats and that can have a strong impact on the optimal foraging strategy for predators that colonize them. First, lack of light makes it difficult to detect prey visually over long distances (Dumas & Chris 1998). Furthermore, in underground environments both predators and prey occur in limited abundance (Uiblein et al. 1992, 1995; Hervant et al. 2000). Therefore in underground environments a meso-predator (i.e. a predator at an intermediate level of the food web) has a low encounter rate with its prey, but also a limited risk of being predated. Theoretical models predict that under these conditions a more active foraging strategy would be more advantageous than the sit-and-wait strategy (Scharf et al. 2006; Hawlena et al. 2011). Both phenotypic plasticity and local adaptations may determine variation for behavioural traits, optimizing foraging strategies in cave fauna (Gordon & Matson 1991; Hervant & Renault 2002; Romero 2009). Understanding the importance of phenotypic plasticity in cave colonization is challenging (Poulson 2011; Romero 2011). Facultative cave dwellers, in which only some populations exploit caves, can be extremely helpful for assessing the relative role of plasticity and local adaptations in the variation of foraging behaviour.

In this study, we compared foraging strategies between cave and epigeous breeding populations of the fire salamander, *Salmandra salamandra*. The fire salamander is usually an epigeous species breeding in streams (Manenti et al. 2009b; Ficetola et al. 2011) but is well able to adapt to new environments (Weitere et al. 2004; Steinfartz et al. 2007; Caspers et al. 2009). Previous studies showed that, in the presence of contrasting breeding habitats, salamander females can show a preference for a specific breeding habitat, and assortative mating can cause genetic differentiation between ecotypes (Weitere et al. 2004; Steinfartz et al. 2007; Caspers et al. 2009). In several areas, the fire salamander often also gives birth to larvae in caves (Manenti et al. 2009a, 2011; Ianc et al. 2012). The peculiar cave features probably expose salamanders to strong selection pressures, and analyses of predatory performance suggest that cave-breeding populations show local adaptations to the underground environments (ecotypes), as they have a better predation performance in complete darkness (Manenti & Ficetola 2013). We used common environment experiments to assess whether phenotypic plasticity or local adaptations enable salamanders to optimize their foraging strategy depending on the environmental conditions. We reared salamander larvae from different origins (cave versus epigeous streams) under contrasting environmental conditions (complete darkness versus normal photoperiod), and tested their behaviour under two light conditions (light versus darkness). We considered two parameters representing foraging behaviour: active search of prey and use of space. First, active search of prey is favoured when prey organisms are scarce, and when they are more difficult to detect (Scharf et al. 2006). If behavioural plasticity determines optimal foraging strategies, then we predicted a more active search for prey in darkness than under the light condition. By contrast, if there are local adaptations, we predicted a different prey search strategy between larvae originating from caves and streams.

Second, in epigeous streams, salamander larvae often remain on the sides of pools, near rocks where they can hide and reduce the risk of being detected by predators (Krause et al. 2011). This risk is lower in caves, where predators are essentially lacking, and in darkness, when predators cannot use visual detection. If variation in space use is determined by behavioural plasticity, we predicted that peripheral sectors will be more frequently used under light conditions. If it is determined by local adaptations, we predicted a higher use of peripheral sectors by stream populations in comparison to cave populations. As foraging strategy may be affected by multiple additional factors (e.g. nutritional status, prey presence, previous experience), we also considered their potential impact. Finally, we evaluated potential ecotype-by-environment interactions, to assess the interplay between plasticity and local adaptation.

METHODS

Study System

We studied fire salamander populations from a karstic area in Lombardy, northwest Italy (approximately 45.8°N, 9.3°E). The area is located between the districts of Lecco and Como and ranges between 340 and 980 m above sea level. The area is characterized by mountainous and hilly reliefs covered by broadleaved woodlands and a dense hydrographic network. In this area, the fire salamander is ovoviviparous and usually gives birth to larvae in streams and creeks (Manenti et al. 2009b; Ficetola et al. 2011). However, there are several natural and artificial caves regularly used for parturition by fire salamanders (see Manenti et al. 2011). These caves are characterized by underground springs which receive water exclusively from the subterranean aquifer; therefore larvae found in caves could not have drifted from superficial water. Females actively enter caves, and select them as breeding sites if they have suitable environmental features (accessibility, presence of prey; Manenti et al. 2009a, 2011). In these environments, salamander larvae are able to develop and metamorphose successfully (Manenti et al. 2011). Salamander larvae are generalist predators feeding on a wide range of benthic invertebrates (e.g. chironomids, culicids, oligochaetes, plecopterans, small larvae of other insects, and small crustaceans; Weitere et al. 2004).

Experimental Setting

We performed a behavioural experiment to assess the foraging strategy of larvae born in hypogeous and epigeous streams under different conditions. We collected newborn larvae (less than 1 week old; larval development stage one: Zakrzewski 1987) from underground springs (11 different caves) and neighbouring epigeous streams (nine different streams). To avoid using related larvae, we used one larva per location. Larvae were transported to the laboratory in separate boxes. In the laboratory, larvae were individually maintained at an average temperature of 18 °C in 10 × 11 cm plastic containers (water depth: 5 cm). Containers with larvae were randomly assigned to one of two different rearing conditions (i.e. giving different experience to larvae): total darkness (light intensity constantly <0.01 lx) and normal day:night photoperiod (12 h light and 12 h darkness). Containers were randomly arranged in two blocks (tanks) per treatment; thus they were not independent. Larvae were fed every second day: 20 live prey items per week during the first 3 weeks and 30 live prey items per week from the fourth week. Prey items were *Tubifex* sp. specimens and chironomid larvae (*Chironomus* sp.) depending on the week.

Behavioural tests started after 45 days of rearing. Each larva was tested under all the combinations of three test conditions: light

conditions (complete darkness versus natural light), presence or absence of prey (one live *Chironomus* larva placed in the opposite side of the container versus no prey); normal feeding versus food deprivation. Food-deprived larvae were maintained for 3 days without food before the test, while the other larvae were fed with five *Chironomus* larvae the evening before the experiment. Food deprivation periods, often fairly long, are likely to happen in natural conditions, especially in caves where trophic resources are extremely rare (Manenti et al. 2009a); thus 3 days without food does not affect larval viability and represents natural conditions. The identity of larvae to be tested each day and the treatments were fully randomly selected; each larva was tested twice under all combinations of treatment conditions (total: 16 tests per larva).

During behavioural tests, each larva was individually placed in a 13.5 × 18.3 cm plastic tank (hereafter called arena) filled with 5 cm of water and allowed to acclimate for 1 min. Each trial lasted 5 min. The tanks were videorecorded with a Nikon easypix NV 500 camera, which can film in both light and complete darkness. Under the darkness condition the camera relied on infrared light which allowed us to see the salamanders (Denoël & Doellen 2010; Manenti & Ficetola 2013) while the salamanders were not able to detect the infrared light (Luo et al. 2011). From the video recordings, we extracted four behavioural parameters: number of explored sectors, average velocity (calculated using video tracking, see below), use of peripheral sectors and, if a prey was present, predation success. The tanks were divided into four interior and four peripheral sectors with equal area (Fig. 1). We recorded the number of explored sectors as the number of times a larva crossed the boundary between two sectors. We distinguished movements toward peripheral sectors from movements toward internal sectors, and calculated the proportional use of peripheral sectors as (number of movements towards peripheral sectors)/(total number of explored sectors; Fig. 1). Videos were then analysed by semi-automated video-tracking procedures using the MTrackJ 1.5.0 plugin (Meijering et al. 2012) in ImageJ 1.46 software (Schneider et al. 2012). Video-tracking systems allow the quantitative and detailed measurement of individual movement patterns (Delcourt et al. 2013; Denoël et al. 2013). Each video was first calibrated to convert pixels into real distances (cm) using reference marks in each video. Locations of salamanders (tip of the snout) were

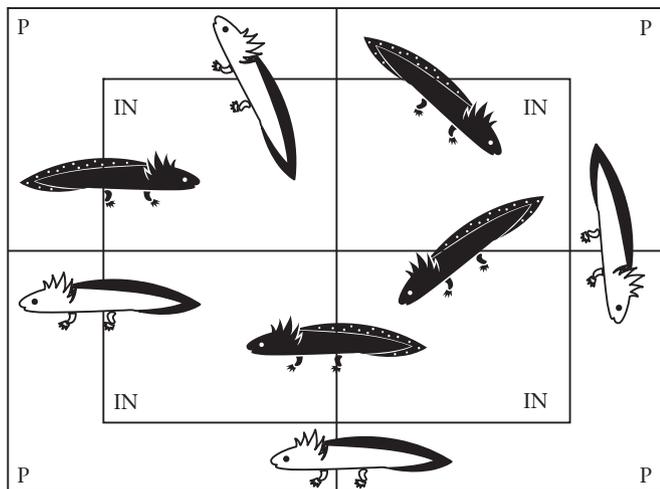


Figure 1. Test arena and example of movements across sectors. The test arena is divided into eight sectors with equal area. Four sectors are peripheral (P), four sectors are internal (IN). White salamanders represent examples of movements towards peripheral sectors; black salamanders represent examples of movements towards internal sectors.

manually determined on fixed images in the video screen by the same observer every 5 s during each 5 min trial for the 320 videorecorded tanks. By using this method, it was possible to obtain all accurate locations of salamander larvae across time, thus avoiding detection losses (Delcourt et al. 2013). We thus obtained 60 spatial coordinates per trial (i.e. 19 200 in total). The analysis of changes in spatial coordinates between each successive analysed image was done automatically by the video-tracking software, which can connect each image in the video sequences (Fig. 2). This gives the distance travelled in each 5 min trial, which was then transformed into average velocity values (cm/s; Denoël et al. 2013).

The collection and maintenance of larvae was in accordance with the Regional Law 10-31/03/2008 (Lombardy Region; permit prot. F1.0002091). After tests, all larvae were released at their site of origin within 1 week.

Statistical Analyses

The average velocity in a trial was significantly correlated with the number of sectors crossed (Pearson correlation between square-root-transformed variables: $r = 0.91$, $P < 0.001$). For this reason, we report analyses of velocity only, as video tracking provides a more objective quantification of larval behaviour (Denoël et al. 2013). Performing analyses by considering the number of sectors crossed as a dependent variable yielded nearly identical results (not shown).

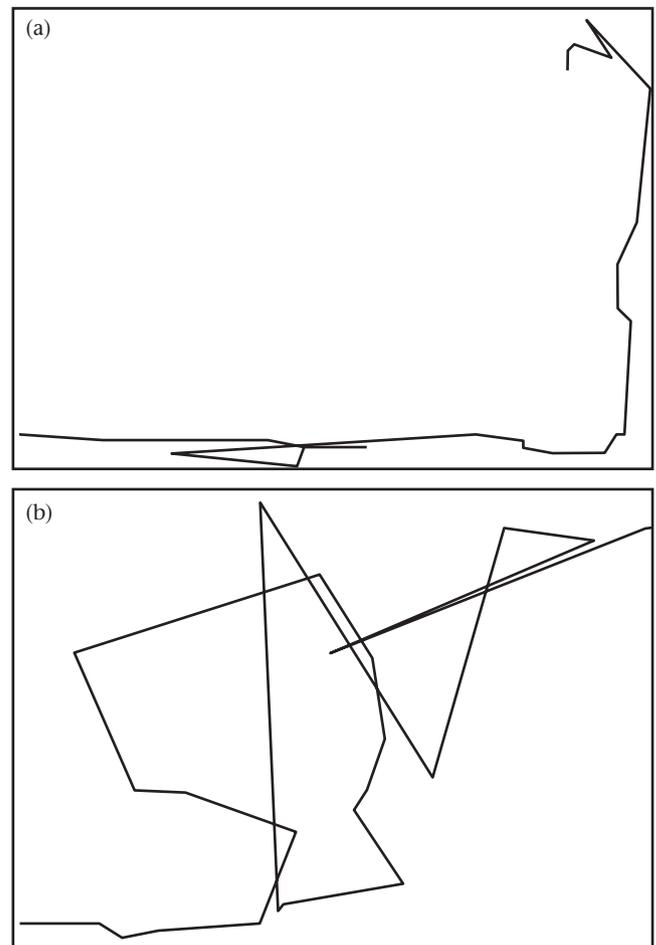


Figure 2. Example of video tracking of the same larva in (a) light and (b) darkness test conditions. (a) Limited movements, mostly in the peripheral sectors; (b) continuous movements exploiting the whole arena. Both tests were performed with a fed larva and in the absence of prey.

We used generalized linear mixed models (GLMM) with Gaussian error to assess the factors determining behavioural parameters of larvae. The GLMM allowed us to analyse dependent variables in which different observations were not independent. In all our models, we included larval identity and rearing block as random factors (Pinheiro & Bates 2000). Furthermore, as some larvae were tested multiple times on 1 day, the trial number on a given day was included as an additional random factor, to take into account potential behavioural modifications in response to repeated tests. In the GLMM, we considered five fixed factors: origin of larva (cave versus stream), light conditions (complete darkness versus light), food deprivation status of larvae (deprived versus fed), prey presence (presence versus absence) during the tests, and rearing conditions (complete darkness versus normal photoperiod) during the 45 days before the tests. Origin of larva was included to test for differences between potential ecotypes, test conditions were included to test for plasticity to contingent conditions, while rearing conditions during the previous 45 days were included to test for long-lasting plasticity. We also tested two-way interactions between fixed factors representing potential non-additive effects between origin, test conditions and rearing conditions (Table 1). The models included the average velocity and the use of peripheral sectors as dependent variables. For each behavioural parameter, the final model included all the fixed factors, plus all the significant two-way interactions. GLMMs (binomial error) were also used to assess the relationships between predation success and measures of larval movements (average velocity, use of peripheral sectors). Only trials with prey presence were considered in this analysis. If needed, variables were transformed using square-root

(velocity) or square-root arcsine (use of peripheral sectors) prior to analysis to reduce skewness and improve normality. Analyses were performed using the lme4 and nlme packages in R (Bates et al. 2011; Pinheiro et al. 2012; R Development Core Team 2012).

RESULTS

The range of average velocity during the 5 min trials was <0.01 cm/s (corresponding to movements <1 cm in 5 min) to 1.46 cm/s (corresponding to a total movement of nearly 4.4 m; average velocity across all tests: 0.34 ± 0.1 cm/s). The average velocity was significantly higher under darkness conditions than in light conditions. Furthermore, food deprivation before the trials and prey availability significantly increased velocity (Table 1, Fig. 3b, c, d). We also detected significant interactions between the origin of larvae and light conditions during tests, between origin and prey presence, and between rearing conditions and light conditions during the test. Under light conditions, and when prey was absent, larvae born in caves reduced activity in light more than those born in streams (Fig. 4, Table 1). Furthermore, larvae reared under day:night photoperiod reduced their velocity under light conditions more than those reared in darkness (Table 1, Fig. 4b).

The use of peripheral sectors was very different between larvae. In 5% of tests, larvae used peripheral sectors only (range of explored sectors 2–28), while in 2% of tests larvae used internal sectors only (range of explored sectors 2–5). Overall, larvae preferred moving in peripheral sectors, as the average use of these sectors was 0.58 (95% CI estimated using 1000 bootstraps: 0.56–0.60; see Fig. 3). Larvae born in streams moved in peripheral sectors more than those from caves (Table 1, Fig. 3f). Furthermore, the average use of peripheral sectors was higher under light conditions than in darkness while, in the presence of prey, larvae moved significantly more towards internal sectors (Fig. 3). None of the interactions between factors was significant for the use of peripheral sectors (Table 1).

The behavioural parameters recorded are relevant for predation performance as, under the same test conditions, predation success was highest in larvae with highest average speed (GLMM taking into account light conditions: $\chi^2_1 = 6.09$, $P = 0.014$) and using the peripheral sectors less ($\chi^2_1 = 5.04$, $P = 0.025$).

DISCUSSION

Salamander larvae showed high plasticity for foraging strategy, as they were able to modify their behaviour in response to environmental conditions. Behavioural modifications were particularly striking between light and darkness conditions: in the darkness the foraging behaviour involved more active movements, with a more balanced use of both peripheral and internal sectors of the arenas. Models predict that the sit-and-wait and the active foraging strategies are favoured under different conditions of prey detectability and abundance (Scharf et al. 2006). The behavioural flexibility observed in this study fits the model expectations well (Scharf et al. 2006; Ioannou et al. 2008; Blumroeder et al. 2012). If plasticity was the dominant process determining adaptation to the cave environments, we predicted that the effect of test conditions on behaviour would offset the effect of origin (i.e. potential ecotypes). This prediction was confirmed for most of the behavioural traits (Table 1), indicating that plasticity has the strongest effect. Nevertheless, significant interactions between origin and test conditions, and a significant effect of origin on the use of peripheral sectors (Table 1), indicate that plasticity is not the only factor taking effect. Local adaptations probably play a role and may even influence plasticity.

Larval salamanders are predators of both invertebrates and small vertebrates and, in environments such as small streams and

Table 1
Results of mixed models relating average velocity and use of peripheral sectors of salamander larvae to larval origin (cave versus stream), light conditions (darkness versus light), food deprivation status (fed versus not fed in the 3 days before the trial), prey presence and rearing conditions (complete darkness versus day:night photoperiod)

Dependent variable	Factor	<i>F</i>	<i>df</i>	<i>P</i>	
Average velocity	Origin	3.62	1,18	0.073	
	Light conditions	11.36	1,148	0.001	
	Food deprivation	18.27	1,148	<0.001	
	Prey presence	7.13	1,148	0.008	
	Rearing conditions	0.01	1,18	0.935	
	Origin*light conditions	11.68	1,148	<0.001	
	Origin*prey presence	10.68	1,148	0.001	
	Rearing conditions*light conditions	4.83	1,148	0.030	
	Nonsignificant interactions (not included in the model)				
	Origin*food deprivation	0.72	1,147	0.396	
	Rearing conditions*origin	0.30	1,17	0.593	
	Food deprivation*prey presence	0.83	1,147	0.364	
	Food deprivation*light conditions	0.01	1,147	0.932	
	Prey presence*light conditions	3.59	1,147	0.060	
	Use of peripheral sectors	Origin	10.71	1,18	0.004
Light conditions		4.44	1,146	0.037	
Food deprivation		1.76	1,146	0.187	
Prey presence		6.96	1,146	0.009	
Rearing conditions		0.29	1,18	0.604	
Nonsignificant interactions (not included in the model)					
Origin*light conditions		0.08	1,145	0.785	
Origin*food deprivation		0.28	1,145	0.597	
Origin*prey presence		1.42	1,142	0.236	
Rearing conditions*origin		2.22	1,17	0.154	
Rearing conditions*light conditions		0.21	1,145	0.647	
Food deprivation*prey presence		0.68	1,145	0.410	
Food deprivation*light conditions		1.31	1,145	0.254	
Prey presence*light conditions		0.60	1,145	0.438	

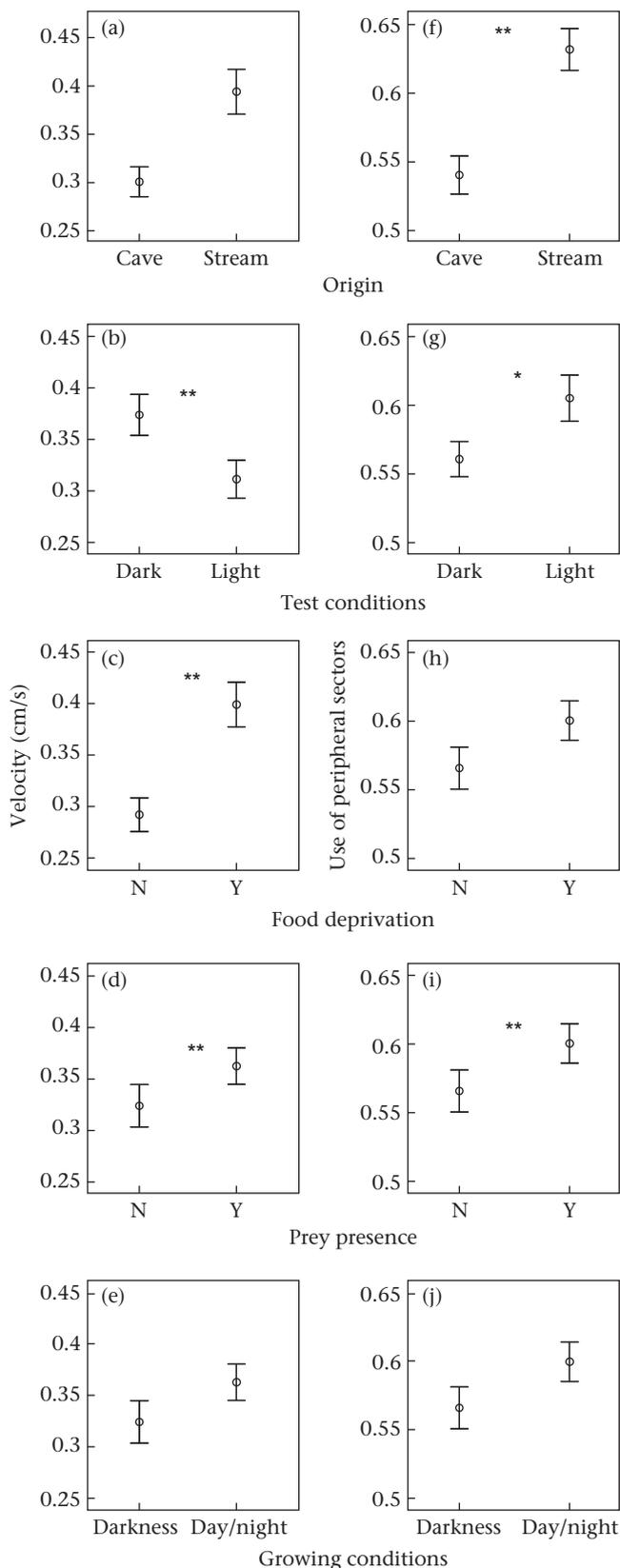


Figure 3. Average behavioural parameters of salamander larvae under contrasting conditions. In (a–e), the dependent variable is the average velocity of larvae during trials; in (f–j) the dependent variable is the proportion of use of peripheral sectors of arenas. Error bars are SEMs. N = no, Y = yes; asterisks indicate significant differences between treatments (* $P < 0.05$; ** $P < 0.01$; see Table 1). Significant interactions are represented in Fig. 4.

underground waters, they are often the top predators (Davic & Welsh 2004). The sit-and-wait behaviour is their most common strategy. Larvae often remain on the substrate and/or hide under stones or vegetation; from these places they make quick attacks on organisms moving close to them (Wells 2007). This strategy reduces the risk of being captured by other predators (e.g. fish, dragonfly larvae), but is only advantageous in the presence of detectable and active invertebrates (Hawlena et al. 2011). As a consequence, plasticity for foraging strategies has been observed in many salamander species. For instance, the patterns of activity can vary in response to prey availability or presence of predators (Taylor et al. 1988; Winandy & Denoël 2013). Distinct foraging strategies have been described in alternative heterochronic morphs in newts (Denoël 2004) and in some obligatory or facultative cave-dwelling amphibian species (Uiblein et al. 1992, 1995). In hypogeous water, prey abundance is much lower than in epigeous environments and the active search favours the localization of nonvisible sedentary or rare prey (Uiblein et al. 1992, 1995).

Food availability and exploitation pose major limits to cave organisms; therefore these factors are expected to exert strong selection pressure and influence foraging (Hervant & Renault 2002). Despite observations that some species of cave-dwelling salamanders tend to have an active foraging behaviour (Uiblein et al. 1992), we are not aware of studies comparing foraging strategies between epigeous and cave-dwelling populations, or exploring the role of plasticity in this framework. Under cave conditions, the scarcity of prey organisms and the difficulty of detecting them are expected to make the standard sit-and-wait strategy less efficient, while lack of predators can reduce the risks associated with higher activity: larvae are thus expected to show less constraint in the use of space, and to use shelters less often.

Our observations are in agreement with both these predictions. In the darkness, salamander larvae from caves had a more active search strategy than in the light condition (Fig. 3), thereby increasing encounter rate (Scharf et al. 2006). This plasticity is certainly important for exploiting underground environments, but may also be useful for larvae living in streams, which are active during both the day and at night (Himstedt 1971). Test conditions also influenced the use of peripheral sectors. In darkness, and with prey present, salamanders exploited the available space more, and did not remain close to the edge of test arenas (Fig. 3g–i). Particularly during daytime, salamander larvae generally remain hidden under stones (Krause et al. 2011; Schauer et al. 2012), and this behaviour is expected to limit the risk of being captured by predators. However, a more complete exploration of the environment increases the likelihood of encountering prey (Ioannou et al. 2008; Winandy & Denoël 2013), particularly if they can only be detected at short distances. Salamander larvae rely on visual, chemical, electric and mechanical stimuli to detect prey (Himstedt 1971; Himstedt et al. 1982). Lack of light reduces the possibility of detecting prey, forcing larvae to get closer before prey detection, but also reduces the risk of being spotted by their own predators. These two factors may act jointly in increasing space use in larvae. In addition, the use of peripheral sectors was significantly lower for salamanders born in caves (Fig. 3f), suggesting local adaptations.

Local adaptation requires some degree of genetic isolation between cave and epigeous populations, but habitat choice and strong selection can maintain divergence even in the presence of substantial gene flow (Via 2009). In fire salamanders, genetic differentiation between ecotypes occurs even in the absence of geographical differentiation. For instance, in central European populations of this species, females can give birth to larvae in two distinct environments: ponds and streams. Local adaptations between pond and stream larvae can be maintained by assortative mating: stream-adapted females show a preference for males from

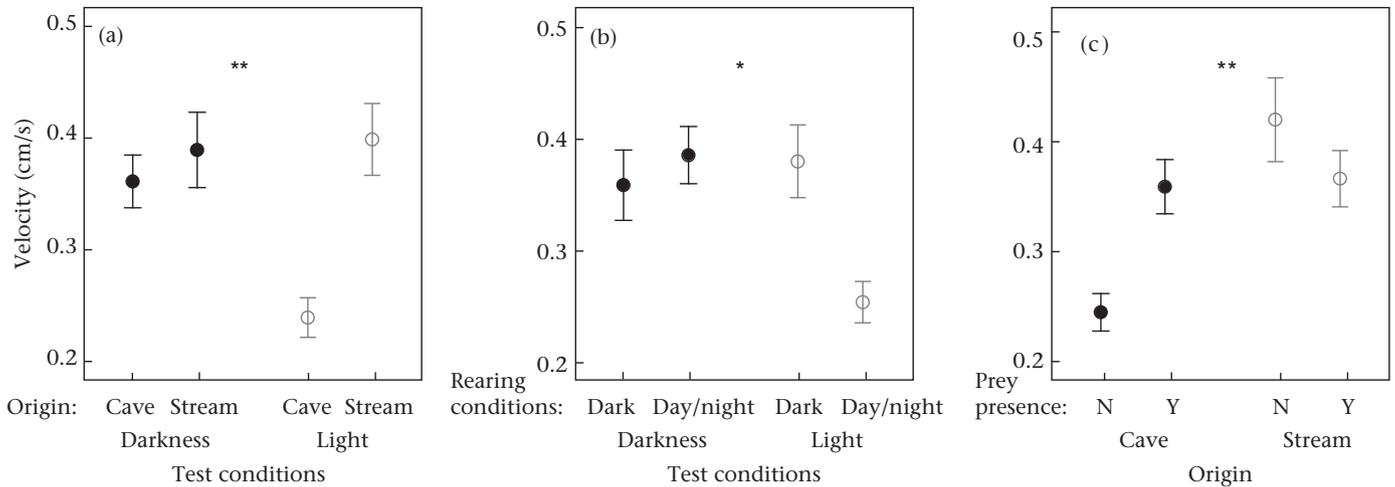


Figure 4. Significant interactions between independent variables on the average velocity \pm SE of larvae during trials. Only interactions significant in Table 1 are represented here. (a) Interaction between origin of larvae and whether the test was carried out in the dark or in the light. (b) Interaction between whether the larvae were reared in the dark or on a day:night photoperiod and whether the test was carried out in the dark or in the light. (c) Interaction between the presence of prey and the origin of larvae. N = no, Y = yes.

the same habitat (Weitere et al. 2004; Steinfartz et al. 2007; Caspers et al. 2009). In our study system, significant differences between cave and stream larvae have been detected across many populations, and in multiple experiments performed on different larvae and during different years (Manenti & Ficetola 2013). Genetic analyses are currently being carried out to ascertain differences between cave and stream populations, although neutral genetic markers may be unable to detect genetic differentiation when gene flow is present and local adaptations are recent (Elmer et al. 2010). Larvae were collected under natural conditions, and it is also possible that some behavioural characteristics may have become fixed early in the development (Ferrari & Chivers 2009). Nevertheless, salamander females give birth to larvae at night, so both stream and cave larvae experienced darkness at the very beginning of their development. Furthermore, larvae were collected at an average age of 3 days (age range 1–6 days), so differences in exposure to light between cave and stream larvae were limited.

Salamander larvae are also able to adjust their risk taking and foraging strategies depending on prey presence and nutrition conditions, and exhibited higher activity in response to prey presence and food deprivation (Fig. 3; Krause et al. 2011). This plastic response is particularly interesting, because it favours active foraging in environments in which prey is scarce, such as caves. As the plastic responses to darkness and food deprivation are in a direction expected to increase fitness (co-gradient variation), it is likely that they can help the colonization of new environments (Crispo 2008). Overall, plasticity was strong in response to test conditions, while the effect of rearing conditions during the previous 45 days was weaker (Table 1). Therefore, salamanders exhibit a highly plastic behaviour, but behavioural modifications are mostly reversible, even if larvae are exposed to specific conditions during essentially their whole lifetime. This observation is in agreement with studies suggesting that plasticity for behavioural traits is reversible, and that modifications are not long lasting (Crispo 2008).

Sit-and-wait and active foraging are two extremes of a behavioural continuum. Within this continuum, a quantitative estimation of the foraging strategy is challenging (Fig. 2): the behaviour of salamanders ranged from no movements at all (1.6% of tests) to larvae moving almost continuously, with average velocity >1 cm/s (3.2% of tests). The behavioural analysis through video tracking allows a quantitative assessment of behaviour. By recording

movements across time, image analysis allowed us to compare locomotion patterns across experimental contexts (Delcourt et al. 2013; Denoël et al. 2013; this study). Salamanders can certainly perform movements for multiple reasons other than foraging. However, we believe that the movements we measured were mostly foraging related. First, velocity affected predation success: salamanders with higher velocity explored more sectors and captured prey more frequently. Furthermore, movements were significantly affected by food deprivation status and prey presence, indicating a relationship between movements, food requirements and food availability. Finally, other potential causes of movements (e.g. search for partners) are unlikely as we analysed the behaviour of larvae.

Plasticity may help colonization and persistence in novel environments through different pathways. First, plasticity can be a strategy for adaptation when selection regimes are alternating, or when high gene flow is present across environmental gradients (Crispo 2008; Crispo & Chapman 2010). Under this scenario, the same generalist genotype may express very different phenotypes depending on the environmental conditions, allowing adaptive responses even in the absence of genotypic differentiation (Neufeld & Palmer 2008; Crispo & Chapman 2010; Gray et al. 2012). Second, plasticity may have a heritable component. The most plastic individuals are the most likely to survive under a wide range of selection pressures, and plasticity is expected to be selected for in populations experiencing the first stages of adaptations. This scenario may determine higher plasticity in the populations adapting to novel environments, initiating or increasing differentiation among ecotypes (Crispo 2008; Torres-Dowdall et al. 2012). These two pathways (i.e. phenotypic plasticity and local adaptation) are not mutually exclusive, and we have found evidence for both. Plasticity in response to the test conditions was high in all larvae. Nevertheless, we observed significant interactions between ecotype and test conditions, as cave larvae reduced activity more when in the light and in the absence of prey (Fig. 4). Such a higher plasticity of cave larvae is expected to improve fitness in the early stages of colonization, and in challenging environments. The ability to modulate movements depending on the conditions can be particularly important in caves, where food shortage is frequent and energy optimization needed.

The importance of plasticity during cave colonization continues to be debated. Some authors have proposed that plasticity plays a

major role in the exploitation of underground environments (Romero 2009, 2011), while others consider it to be a marginal phenomenon (Poulson 2011; Pipan & Culver 2012). Our study indicates that it may be difficult to tease apart the role of plasticity and local adaptations, as the interplay between these processes can be complex, and there are situations in which both together play an important role. Plasticity for behavioural traits might be the dominant process at the early stages of colonization, and may permit the diversification necessary to exploit new ecological niches, thereby promoting adaptive divergence at the subsequent steps (Crispo 2007).

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