Environmental factors determining growth of salamander larvae: A field study

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Abstract Larval growth and survival of organisms are strongly influenced by abiotic and biotic factors, as demonstrated by experimental studies performed under controlled laboratory or semi-natural conditions. Even if they have many advantages, experiments cannot cover the full complexity of natural conditions and field studies are needed for a better understanding of how environmental variation determines growth and development rate. Fire salamander Salamandra salamandra females give birth to larvae in a variety of habitats, both epigean and subterranean. In caves, salamander larvae successfully grow and metamorphose, but their growth is more than three times longer than in epigean streams and factors determining these differences require investigation. We performed a field study to understand the factors related to the growth of fire salamander larvae in different environmental conditions, evaluating the relationship between environmental features and larval growth and differences between caves and epigean spring habitats. Both caves and epigean larvae successfully grew. Capture-mark-recapture allowed to individually track individuals along their whole development, and measure their performance. Growth rate was significantly affected by environmental variables: larvae grew faster in environments with abundant invertebrates and few conspecifics. Taking into account the effect of environmental variables, larval growth was significantly lower in caves. Food availability plays a different effect in the two environments. Larval growth was positively related to the availability of invertebrates in epigean sites only. The development rate of hypogeous populations of salamanders is slower because of multiple parameters, but biotic factors play a much stronger role than the abiotic ones [Current Zoology 61 (3): 421–427, 2015].

Keywords Spring, Freshwater, Cave, Amphibians, Salamandra, Cannibalism

The rate of growth, development and survival of organisms may be strongly influenced by multiple abiotic and biotic factors. For instance, high temperature is often associated with faster growth and development in many ectotherms (Angilletta and Dunham, 2003; Ringia and Lips, 2007; Couret et al., 2014); food availability and nutrient quality strongly influence survival and growth rate (Denoël and Poncin., 2001; Kingsolver and Huey, 2008; Couret et al., 2014), and intraspecific competition may determine delayed maturity even in absence of food shortage (Angilletta and Dunham, 2003; Relyea and Hoverman, 2003; Kingsolver and Huey, 2008; Legros et al., 2009; Ficetola et al., 2011; Couret et al., 2014). The importance of abiotic and biotic conditions on individual growth rate has been demonstrated by multiple studies, showing that the relative importance of these parameters may be highly variable across species and study systems. Nevertheless, the majority of studies have been performed under controlled laboratory or semi-natural conditions. Studies under controlled conditions have many advantages, such as replication level, complete control on environmental parameters and on the study individuals. However, laboratory studies sometimes do not cover the full complexity of natural conditions, and it may be difficult linking the output of controlled condition experiments with the performance of populations in the wild (Carpenter, 1996; Skelly and Kiesecker, 2001; Skelly, 2002). Field studies are therefore needed for a better understanding of how environmental variation determines changes in performance, and which parameters are the most important in influencing growth and development rate (Denoël et al., 2007; Whiteman et al., 2012). Nevertheless, logistic constraints have somehow limited the organization of
detailed studies under natural conditions. For instance, an accurate measurement of individual growth rate may require the repeated capture and identification of many individuals over multiple occasions and in heterogeneous environment sites.

Studying species able to exploit multiple environments is extremely important to evaluate how environmental conditions influence individual performance in the wild. A useful environment to be considered are caves. Many amphibians, especially Urodels, can exploit underground habitats during their life cycle. Apart from troglobions, that spend their entire life in caves, there are several species of amphibians whose populations can be more or less regularly linked to caves or other subterranean environments, and several of them occasionally breed underground (Bressi and Dolce, 1999; Ringia and Lips, 2007; Manenti et al., 2009a; Manenti et al., 2011). In these habitats amphibians can take advantage of limited or absent predators, stable hydroperiod and a prolonged reproductive season, but they have to face difficulties such as limited food supply and lack of light (Ringia and Lips, 2007; Manenti et al., 2009a). Clergue-Gazeau (1975) hypothesised that cave environment strongly affect Urodels growth and development, by increasing the length of larval growth and retarding metamorphosis.

Even if the fire salamander *Salamandra salamandra* lives generally in epigean sites, it is an urodele that can be found in underground habitats, especially at larval stages, as females give birth to larvae in a variety of habitats, including pools, streams, springs and other waterbodies, both epigean and subterranean (Weitere et al., 2004; Manenti et al., 2009b; Romano et al., 2010; Schauer et al., 2012). In caves the larvae successfully grow and metamorphose, and there are evidences of behavioural adaptations to subterranean habitats (Manenti et al., 2013a; Manenti and Ficetola, 2013). On the other hand, larval development of underground populations is more than three times longer than in larvae living in nearby, epigean streams (Manenti et al., 2011), but the factors determining these development differences require investigation.

Larval growth and development rate are major parameters determining fitness in amphibians (McDiarmid and Altig, 1999), therefore the identification of factors influencing larval growth may provide new insights on the performance in both epigean and hypogeous habitats. Parameters that may influence growth rate of salamander larvae include temperature (Ringia and Lips, 2007), food availability (Denoël and Poncin., 2001), intraspecific competition and the presence of predators like fish (Munshaw et al., 2014). Both in caves and in streams, salamander larvae are often laid in small pools where food is scarce and competition is high (Manenti et al., 2009b). The abundance of invertebrates and of conspecifics appear therefore as major biotic variables that could influence the growth rate of larvae. Among the abiotic factors, both physical (e.g. light, temperature) and chemical (e.g. pH) parameters may affect larval growth (Swierad and Zakrzewski, 1990; Griffis-Kyle and Ritchie, 2007). In this study we focused on light and water temperature. Salamander larvae are predators and their predatory strategy is strongly influenced by light availability (Manenti et al., 2013a), although olfactory cues may also be important for both larval and adult fire salamanders (Caspers and Steinfartz, 2011; Ibáñez et al., 2014). Light availability is therefore expected to influence larval development. Finally, temperature strongly influences the metabolism of ectothermic organisms. Therefore, in amphibians growth is generally lower in populations living in cold environments, such as those living in caves (Clergue-Gazeau, 1975; Ringia and Lips, 2007). All these factors can strongly differ between hypogeous and epigean sites, and may cause the development delay observed in larvae living underground. The aim of this study was identifying the factors related to the growth of salamander larvae in a variety of environmental conditions. Specifically, we 1) evaluated the relationship between environmental features and the fire salamander larval growth and 2) assessed if this relationship changes across populations living in strikingly different environments, such as caves and epigean habitats.

1 Material and Methods

1.1 Study areas and surveys

We surveyed 12 caves and 4 epigean springs in northwest Lombardy (NW-Italy) respectively from February to August 2013 and from May to October 2013. Each site was monitored monthly (total: 6 surveys for cave springs and 9 surveys for epigean springs). Caves are situated in the southern part of the so called “Larian Triangle”, an area delimited by Como, Lecco and Monza districts between 200 and 850 m a.s.l.; this area is characterized by hilly and mountainous reliefs covered by mixed broadleaved woods. We considered both natural and artificial caves as draining galleries (horizontal tunnels that penetrate the side of a slope) and typical “bottini” (small buildings with basins for water catching). Fire salamander reproduction is fre-
quent in these cavities, and salamanders are able to successfully complete larval development in these environments (Manenti et al., 2009a; Manenti et al., 2011). Epigean springs are situated in Valzurio municipality (Bergamo district, Orobie Prealps) between 1,200 to 1,500 m a.s.l and are characterized by small natural pools filled by slow running water emergences (approx. coordinates of the center of the study area: 45.5° N E9.5° E).

1.2 Field surveys

In each site, larvae were captured using a small dip net (mesh size: 1 mm). Catching was performed until any larva was visible in the site, and was continued for at least 10 min. after capturing the last larva. Larvae were maintained in a small aquarium (20×10 cm, depth 5 cm), and the capture session was repeated 30 min later. For each larva, we recorded total length (accuracy: 1 mm) and weight (accuracy: 0.01 g). Furthermore larvae were photographed laterally with a Casio Exilim Ex H30 camera, following the recommendation of Eitam and Blaustein (2002) to allow individual identification.

At each survey, we recorded four biotic and abiotic environmental parameters that are potentially important for fire salamander larvae. Two abiotic factors were recorded using a CEM DT8820 multiparameter: maximum illuminance incident on the pool surface and water temperature. Furthermore, as biotic parameters we considered a measure of intraspecific competition (abundance of fire salamander larvae) and a measure of food availability (biomass of invertebrates). The abundance of salamanders for each site and sampling session was estimated on the basis of the two successive removal samplings, applying the removal method proposed by Chao and Chang (1999). The abundance of invertebrates was estimated through pipe sampling (Dodd, 2010). Samples were collected by thrusting a 0.3 m² circular pipe sampler through the water column and about 5 cm into the sediment. Small nets (mesh size: 1 mm) were used to remove all animals from the water and the first cm of the sediment (Dodd, 2010). Net sweeps were collected until at least 10 consecutive empty sweeps; for each site we repeated pipe sampling twice and then weighed in loco all the organisms collected with a Shun Yuan MH-200 field balance, precision 0.01g. We then calculated the wet biomass of invertebrates for each site (g/m²). Larvae were individually identified on the basis of pictures, following the Eitam and Blaustein’s method (Eitam and Blaustein, 2002). For each larva, we calculated growth rate as the average daily increment in weight between two successive captures (weight t₁ - weight t₀)/ (date t₁ - date t₀); growth rate values are in in milligrams per day. The mean interval between captures was 25 days.

1.3 Statistical analysis

We used a t-student test to test the significance of differences between the environmental factors of caves and streams. We used linear mixed models to assess the factors determining larva growth, because they allowed us to analyze variables in which different observations were not independent. In our model we included site, larval identity and survey as random factors. To evaluate whether differences between caves and springs hold when taking into account environmental differences, we considered both environmental features (abundance of invertebrates, abundance of salamander larvae, water temperature, illuminance) and site typology (cave/stream) in the same model. Given our large sample size compared to the number of predictors, we built one single model considering all the environmental variables. We also tested all the possible interactions between site typology and the four environmental variables; only significant interactions were included in the final model. Variables were checked for normal distribution (Shapiro test) and if needed transformed using logarithm (illumiance). We used a Likelihood-ratio based pseudo-R² to assess the amount of variation explained by our mixed model (Nagelkerke, 1991). All the analyses were performed in R 3.01 environment using the nlme, car, vegan and MuMin packages.

2 Results

Underground sites showed lower average illuminance (t-test: \( t_{14} = 7.650; P < 0.001 \) (Fig.1)) and lower prey density (\( t_{14} = 7.253; P < 0.001 \)) than outdoor springs, while differences in temperature were not significant (\( t_{14} = 1.886; P = 0.08 \) (Fig.1)).

Overall, we performed 3,160 captures during the surveys. 136 larvae were identified through the analysis on photographs in more than one capture session. Generally the average recapture rate was of 56.1% (±1.8%) in caves and 49.8% (±3.2%) in epigean springs. The abundance of larvae was highly variable among sites and along time. Maximum abundance per site ranged between four and 148 individuals, but abundance showed strong temporal variation for all sites. Standard errors of abundance estimates were limited, suggesting reliability of our abundance estimates. Average abundance was significantly lower for cave populations (\( t_{14} = 4.709, P < 0.001 \)).

Larvae grew successfully in both underground pools and epigean springs. Average growth rate was 1.6–8.3
mg/day for epigean springs (mean: 4.0 mg/day), while it was 0.6–12.0 mg/day for caves (mean: 3.30 mg/day). Differences in growth rate between the two environments were not significant (linear mixed model: $F_{1,88} = 0.03, P = 0.86$). Growth rate was significantly affected by environmental variables: larvae grew faster in environments with abundant invertebrates and few conspecifs (Fig. 2A). After taking into account the effect

![Fig. 1](image1.png)

**Fig. 1** Box plot showing differences between cave pools and epigean springs for abiotic and biotic variables

![Fig. 2](image2.png)

**Fig. 2** Partial regression plots showing relationship between growth rate and A) abundance of salamanders per site; B) abundance of invertebrates

In the panel B, black points / black lines represent larvae from cave populations, while grey points / grey lines represent larvae from springs. Shaded areas are 95% confidence intervals. The abundance of salamanders was transformed prior to perform analysis using square-root, therefore the relationship appears non-linear with the untransformed x-axis.
of environmental variables, larval growth was significantly lower in caves (Table 1). Furthermore, we detected a significant interaction between site typology (cave/stream) and invertebrate biomass (Table 1), indicating that food availability plays a different effect in the two environments. Specifically, larval growth was positively related to the availability of invertebrates in epigean sites (linear mixed model: $F_{1,102} = 7.00, P = 0.01$), but not in caves ($F_{1,90} = 1.73, P = 0.19$; Fig. 2B). The pseudo-$R^2$ value of the model was 0.13.

Table 1  Linear mixed models analysis assessing factors determining larval growth

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertebrate biomass</td>
<td>5.70</td>
<td>1,203</td>
<td>0.018</td>
</tr>
<tr>
<td>Salamander abundance</td>
<td>23.04</td>
<td>1,88</td>
<td>&lt;0.001</td>
</tr>
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<td>Temperature</td>
<td>0.28</td>
<td>1,203</td>
<td>0.600</td>
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<tr>
<td>Illuminance</td>
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<td>1,203</td>
<td>0.273</td>
</tr>
<tr>
<td>Site typology (epigeous/cave)</td>
<td>0.53</td>
<td>1,88</td>
<td>0.467</td>
</tr>
<tr>
<td>Typology × invertebrate biom.</td>
<td>7.08</td>
<td>1,203</td>
<td>0.008</td>
</tr>
</tbody>
</table>

3 Discussion

Habitat features and trophic resources strongly affect the development and the early life history in amphibians (Wells, 2007). Our study reveals that the examined biotic features play the major role on the growth of larvae, while the effect of abiotic environmental characteristics was weaker. The level of illuminance was clearly completely different between epigean and hypogeous habitats, while differences for water temperature were minor (Fig. 1). Temperature is a major determinant of amphibian metabolism and developmental processes (Wells, 2007) and for this specific features mountain epigean spring pools and cave pools do not differ particularly, as already supposed (Ringia and Lips, 2007). Nevertheless, in mountain springs temperature shows more variability. Prey density is significantly higher in pool springs than in cave pools, confirming previous reports of limited food supply for fire salamander larvae in caves (Manenti et al., 2009a; Manenti et al., 2011). Larval growth was positively related to the biomass of invertebrates, confirming that rich nutrient contexts increase larval size and weight (Krause et al., 2011).

The abundance of conspecific larvae was the parameter most strongly related to growth rate, with a much slower growth when abundance was high (Fig. 2A). Abundance of conspecifics may influence growth rate through multiple processes. First of all, more conspecifics mean more competition for limited resources, such as food (Relyea and Hoverman, 2003); furthermore, high abundance of conspecifics means more intraspecific interactions, which may negatively affect larval growth. For instance, at high density salamander larvae show frequent aggressive interactions (Reques and Tejedo, 1996) and the largest size classes of larvae often prey on young-of-the-year larvae (Wissinger et al., 2010).

Food scarcity and high density of conspecifics are frequent in hypogeous environments, so these parameters likely determine the slower growth rate in these environments, as observed in studies on cave populations of fire salamander (Gimenez-Lopez and Guarner Deu, 1982; Manenti et al., 2011). In our study, the highest abundance of larvae was registered in epigean pools (Fig. 1D), while prey density was lower in the hypogeous ones. Food availability has a different role between caves and epigean environments. Growth rate was positively related to the abundance of invertebrates, but in epigean springs only, where the prey density is significantly higher. Conversely, in caves, where available prey are scarce, we found no significant relationship between abundance of invertebrates and growth rate. There are different explanations for these differences. First, populations in different habitats may exploit different food resources. Benthonic invertebrates are the main food of salamander larvae in epigean streams (Reinhardt et al., 2013). However, the limited abundance of macrobenthos in caves might cause a shift of diet. For example there are different small crustaceans like ostracods or small molluscan gastropods that often inhabit underground environments (Pipan et al., 2008; Pezzoli, 2010) which detection requires specific techniques and have not been sampled in the present study. Moreover coprophagy has been described for some cave populations of salamanders (Fenolio et al., 2006) and recent studies showed that larvae in ponds can feed on large amounts of terrestrial invertebrates (Reinhardt et al., 2013). The consumption of organic detritus or dead insects fallen in the pools may not be excluded. A role of cannibalism is also possible (Kohmatsu et al., 2001; Wildy et al., 2001). Complete cannibalism has been reported from multiple salamander species when disparities between larvae sizes are strong (Wells, 2007), thus it is likely that old larvae from former cohorts prey upon newly laid larvae. Finally, larvae density may increase aggressiveness (Reques and Tejedo, 1996) and it is likely that also starving conditions do. Thus the scarcity of food may increase the importance of cannibalistic behaviour in underground sites.

This field-analysis is the natural continuation of pre-
vious studies performed in controlled laboratory conditions and it partially confirms the Clergue-Gazeau’s hypothesis (Clergue-Gazeau, 1975), suggesting that the development rate of hypogeous populations of salamanders is slower because of multiple parameters, among which scarcity of prey. The number of epigean sites investigated was limited; other factors likely affect larval growth of fire salamanders, such as water chemistry and predator occurrence. Further studies considering other typologies of epigean breeding sites may allow to disentangle the role played by predator occurrence like dragonfly larvae (Manenti et al., 2013b) and to assess differences between caves and epigean habitats. The success of larvae development in both environments, as evidenced by our study, further underlines the great plasticity of this salamander, that makes it able to colonize a large variety of habitats. Understanding the environmental features affecting larval growing in subterranean populations of a usually epigean species like the fire salamander can provide useful insights for the planning of further studies focusing on the processes favouring cave exploitation and colonization by non-strict cave-dwelling organisms.

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References


