Development of the fire salamander larvae at the altitudinal limit in Lombardy (north-western Italy): effect of two cohorts occurrence on intraspecific aggression

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Abstract. During 2013, we surveyed the larval population of the fire salamander that inhabits different pseudo-artificial headwater pools in Lombardy, Italy. To understand altitude effects on breeding, larval development and intraspecific aggression, we focused on sites at the altitudinal limit for the spawning of the species in this region (around 1491 m a.s.l.). Sites have been visited two times per month between spring and autumn; in each pool, larvae were collected, weighed, measured and individually recognized. We assessed the density of larvae and the frequency of injured ones. Through pipe-sampling we monitored prey biomass and density. Our study verifies that two cohorts of fire salamander larvae exist in the pools at the altitudinal limit in Lombardy. Moreover, we found the deposition of two eggs with abortive embryos inside. Larvae that were laid in April 2013 reached a total length of 47.0 to 62.5 mm by October. Pools hosted high prey density, but low biomass. The frequency of injured larvae found was generally higher than reported for caves and lower than observed in cases of high predation pressure by Odonata larvae. The deposition of abortive eggs represents the first record of this phenomenon for the populations of fire salamander of the Pre-Alps. Generally, our study provides new data on fire salamander larvae growing at high altitudes.

Keywords: Salamandra salamandra, larval development, abortive embryos, Italy.

Introduction

Amphibians are ectothermic organisms, particularly sensitive to the effects of altitudinal and topographic variation for different reasons (Wells 2007). As the dispersal and distribution of amphibians is generally conditioned by the occurrence of moist suitable terrestrial habitats (Graeter et al. 2008), the damp site colonization in mountain areas, where dry lines often occur, may be limited (Rothermel & Luhring 2005, Wahbe et al. 2005). Moreover, altitude may affect larval growth (Denoël & Poncin 2001). In mountain pools, UVB radiation is often quite strong during daytime and the larval mass and length are reduced by exposure to intensive radiation (Blaustein et al. 2005, Scheessele 2007). Temperature often plays a key role in determining complex growth as well as developmental phenotypes and mountain damp biotopes are considered as cool and poor productive environments. Therefore, larval growth and other life history traits are usually strongly affected (Ringia & Lips 2007).

The fire salamander Salamandra salamandra (L., 1758) is a widespread amphibian throughout Europe (Sillero et al. 2014), which typically inhabits deciduous mixed woodlands and breeds in different water body typologies (Manenti et al. 2009b). In the Pre-Alps, the typical breeding waters are headwaters with clear, cool waters rich in oxygen content. However, some populations use standing waters for breeding, such as ephemeral wetlands, pools used for livestock watering or irrigation, and even cave habitats, displaying a remarkable ecological plasticity with surprising local adaptations (Weitere et al. 2004, Manenti et al. 2011, Manenti & Ficetola 2013). In these fish-free aquatic environments, fire salamander larvae may be the top vertebrate predator, altering entirely the food web linkages between the ponds and the adjacent terrestrial habitats (Reinhardt et al. 2013).

Fire salamanders represent an outstanding example of intraspecific polymorphism (Buckley et al. 2007, Velo-Anton et al. 2007, Velo-Anton et al. 2012). This applies for both morphological and developmental features, and variation was also observed in the reproductive strategy (Buckley et al. 2007). Alternatively to the normal ovoviviparous condition, viviparity occurs. In the case of some populations of the subspecies S. s. fastuosa,
which lives at 1000 m a.s.l., females lay a few giant larvae of stages close to metamorphosis into water (Joly 1986). In the case of *S. s. bernardezi*, females lay completely metamorphosed young specimens (Velo-Anton et al. 2012). In both of these subspecies, larvae feed on abortive eggs or embryos in the female uterus (Joly 1968).

Another plastic life history trait of fire salamanders is the duration of the larval period.

In amphibians, nutrient availability and food quality strongly influence survival and growth rate (Denoël & Poncin, 2001); growth is generally lower in populations living in cold environments such as caves (Clergue-Gazeau 1975, Ringia & Lips 2007). In the fire salamander, the larval growth may extend from few months in usual eugeous breeding sites to more than one year as it occurs in caves (Manenti et al. 2009a) or in the high altitude ponds exploited by *S. s. almanzoris* (Guerrero et al. 1990). In northern Italy, the fire salamander usually shows two main peaks of deposition, one in the spring and the other in the autumn (Giovine 1996). Larvae laid in autumn, usually overwinter and metamorphose in the spring of the successive year (Giovine 1996). The larvae of the autumn cohorts likely feed on spring newborn larvae (Giovine 1996, Reques & Tejedo 1996).

The altitudinal distribution of subspecies of fire salamander in Italy ranges from the sea level in the northern parts of its range to 2000 m a.s.l. In the northern Alps, the fire salamander is widely distributed at altitudes between 200 and 700 m a.s.l. and it is also found at up to 1300 m a.s.l. in Austria and Switzerland (Grossenbacher 1988, Schauer et al. 2012). In the southern Alps it occurs up to 1700 m a.s.l. in Piedmont (Di Cerbo & Razzetti 2004). On the Cancervo Mountain in Lombardy, there is an observation of an adult salamander that was found at 1800 m a.s.l., but successive enquiries revealed that the reported altitude is erroneous (G. G. pers. obs.). Prior to our study, the recognized altitudinal limit of the species’ breeding sites in Lombardy was 1400 m a.s.l. (Di Cerbo & Razzetti 2004). In April 2012 two of us (G. G. and R. M.) discovered a series of springs used as breeding sites by *S. salamandra* in the Orobian Pre-alps in Lombardy (north-western Italy), the uppermost being at 1491 m a.s.l. which exceeds the recognized altitudinal limit of the species in this region. In 2013, we performed an extensive survey on larvae growing in these sites with the aim to understand the duration of larval growth and to assess metamorphosis and laying periods.

**Material and methods**

The study area is located near Bergamo, within the Site of Community Importance number: IT2060009 (named “Val Sedonia, Val Zurio e Pizzo della Presolana”). In the winter of 2012–2013, the area had an average minimum temperature of ~0.1 °C with a peak of ~4.4 °C in January and average total precipitations of 188 mm (data from the close meteorological station of Zumla). We monitored four spring pools situated between 1160 and 1491 m a.s.l. (Table 1). The pools are relatively small (surfaces vary from 9.3 to 1.48 m²) with low depths (average 13 cm) and no aquatic vegetation cover except for *Spirogyra* sp. algae (Fig 1). They occur in an ecotone habitat among beech forests and pasture lands. After the discovery of the sites in May 2012, we performed a preliminary investigation in April 2013. In all the pools, there were several new-born larvae, and in three pools, larvae that likely overwintered. From May to October 2013, we performed regular surveys every two weeks. At each survey, we recorded four abiotic environmental features that are supposed to be important for fire salamander larvae for every pool: area, maximum depth, water temperature (at 5 cm of depth) and maximum illuminance using a CEM DT8820 multi-parameter (PCE instruments). As a biotic parameter, we recorded the biomass of macroinvertebrates using the pipe-sampling technique. For every pool, we performed at least two samples using a 0.3 m² circular pipe sampler.

![Figure 1. The studied spring pools (photo by G. R.).](image-url)
Table 1. Data for the four different pools (UTM coordinates based on WGS84 datum, altitudes, area) and invertebrates found herein.

<table>
<thead>
<tr>
<th>Pool</th>
<th>N</th>
<th>E</th>
<th>Altitude (m a.s.l.)</th>
<th>Area (m²)</th>
<th>Invertebrate biomass (g)</th>
<th>Invertebrate density (g/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1576388</td>
<td>5087678</td>
<td>1491</td>
<td>1.48</td>
<td>0.08</td>
<td>70.1</td>
</tr>
<tr>
<td>B</td>
<td>1576346</td>
<td>5087704</td>
<td>1485</td>
<td>9.3</td>
<td>0.05</td>
<td>70.7</td>
</tr>
<tr>
<td>C</td>
<td>1576505</td>
<td>5087415</td>
<td>1420</td>
<td>2.19</td>
<td>0.04</td>
<td>42.1</td>
</tr>
<tr>
<td>D</td>
<td>1576116</td>
<td>5086968</td>
<td>1160</td>
<td>1.75</td>
<td>2.14</td>
<td>259</td>
</tr>
</tbody>
</table>

that we put into the sediment. We removed all the macrobenthos from the sampler water column using a fine mesh net (mesh size 1 mm) (Dodd 2010). Net sweeps were collected until at least 10 consecutive empty sweeps occurred; for each site, we repeated pipe sampling twice and then weighed all the organisms collected. We then calculated the wet biomass of invertebrates for each site (g/m²). Larvae were individually identified on the basis of pictures, following Eitam & Blaustein's method (Eitam & Blaustein 2002). At each sampling, we recorded the total length and the weight of the larva. For each recognised larva, we calculated growth rate as the average daily increment in weight between two successive captures. For every pool, we estimated the larval density (the abundance of salamander larvae) by performing two successive sampling sessions and applying the removal method proposed by Chao & Chang (1999). We examined every larva to detect injuries and then calculated the frequency of injured individuals as proportion of the total observed larvae; this injury frequency is a useful proxy of conspecifics aggression (Munshaw et al. 2014). Differences between frequencies were assessed using a Wilcoxon non parametric test.

Results

Between May and October 2013, we performed 2493 captures of at least 318 different larvae. 93 larvae that were captured during the first survey were found in the last one. All the pools hosted suitable invertebrate prey (Table 1). The most representative items were the Chironomidae: Tanytarsus sp., Prodiamesa olivacea, Procladius choreus and Micropsectra notescens. Chironomidae reached collectively a maximum density of over 700 individuals/m² (average density 253 ± 92 individuals/m² (CI 0.95)).

In all pools, larvae successfully grew (Tables 2, 3). Larvae that overwintered between 2012 and 2013 were 50 mm long in May and reached the 3A and 3B pre-metamorphic stages (Juszczyk & Zakrzewski 1981) in July (total length close to 60 mm).

Larvae that were laid in April 2013 reached a total length of 47.0–62.5 mm by October.

The frequency of injured larvae was lower in spring (W = 743.2; p < 0.001) when larvae density was higher (W = 167; p < 0.01), but their length was shorter (W = 45; p < 0.01); the frequency was maximum in autumn (Fig. 2, 3). The average frequency was higher in pool D (22%) and in pool A (25%), where a Cordulegaster larva was found that may have preyed upon fire salamander larvae.

Discussion

Our study verified that two cohorts of fire salamander larvae exist in the pools at the altitudinal limit in Lombardy. Larvae laid in April that do not reach metamorphosis, overwinter and meta-
Development of the fire salamander larvae

Table 2. Average total length (TL) and weight of larvae laid in April 2013.

<table>
<thead>
<tr>
<th>Pool</th>
<th>Average TL in early May</th>
<th>SE</th>
<th>Average TL in October</th>
<th>SE</th>
<th>Average weight in early May</th>
<th>SE</th>
<th>Average weight in October</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35.21</td>
<td>3.24</td>
<td>52.53</td>
<td>5.83</td>
<td>0.31</td>
<td>0.06</td>
<td>0.88</td>
<td>0.19</td>
</tr>
<tr>
<td>B</td>
<td>35.39</td>
<td>2.28</td>
<td>60.28</td>
<td>2.71</td>
<td>0.34</td>
<td>0.05</td>
<td>1.37</td>
<td>0.22</td>
</tr>
<tr>
<td>C</td>
<td>34.71</td>
<td>6.14</td>
<td>50.03</td>
<td>3.45</td>
<td>0.28</td>
<td>0.06</td>
<td>0.88</td>
<td>0.13</td>
</tr>
<tr>
<td>D</td>
<td>31.44</td>
<td>4.39</td>
<td>-</td>
<td>-</td>
<td>0.24</td>
<td>0.07</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. Average total length (TL) and weight of larvae that overwintered between 2012 and 2013.

<table>
<thead>
<tr>
<th>Pool</th>
<th>Average TL in early May</th>
<th>SE</th>
<th>Average TL in July</th>
<th>SE</th>
<th>Average weight in early May</th>
<th>SE</th>
<th>Average weight in July</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>52.49</td>
<td>0.56</td>
<td>58.97</td>
<td>0.43</td>
<td>1.04</td>
<td>0.23</td>
<td>1.40</td>
<td>0.26</td>
</tr>
<tr>
<td>B</td>
<td>51.31</td>
<td>0.89</td>
<td>62.21</td>
<td>0.76</td>
<td>1.03</td>
<td>0.19</td>
<td>1.76</td>
<td>0.31</td>
</tr>
<tr>
<td>C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>48.94</td>
<td>1.02</td>
<td>52.33</td>
<td>0.91</td>
<td>0.67</td>
<td>0.21</td>
<td>0.90</td>
<td>0.15</td>
</tr>
</tbody>
</table>

morphose in June–July of the next year. Moreover, we found that two main periods of deposition occurred at the species’ altitudinal limit, one in the spring and the other in the autumn. Overwintering is a common pattern of larvae laid in autumn (Giovine 1996). Metamorphosis took at least six months for larvae laid in spring.

Trophic resource availability has been shown to be one of the most important environmental features during the early life phase of several vertebrates (Criscuolo et al. 2008). Krause et al. (2011) showed that when larvae of S. salamandra experience rich nutritional conditions during the first three months of life, then they become heavier, larger and are in a better overall condition, compared to larvae that experienced poor nutritional conditions. Moreover, individuals that have food available, complete metamorphosis much earlier (Krause et al. 2011). In our mountain pools, even if abundant, the available prey had generally a low biomass. However, growth duration until reaching the pre-metamorphosis stages 3A and 3B (Juszczyc & Zakrzewski 1981) was shorter than in caves (Manenti et al 2011). Caves, together with mountain freshwater habitats, are usually considered food deprived environments (Ringa & Lips 2007). The long duration of the larval growth is a disadvantage of breeding at higher altitude (Johnson et al. 2006). At lower altitudes, where larval development may be completed in 3–4 months, young metamorphosed individuals may feed in the terrestrial habitat and reach bigger sizes before winter, enhancing their probability to survive (Krause et al. 2011). On the other hand, climatic conditions in valleys situated at higher altitude may be more favourable during hot and dry summer periods. Also, the role played by the presence of lentic habitats on fire salamander occurrence in mountain environment should be attentively considered. Lotic environments at high altitudes may be too torrential and unsuitable to allow breeding and larval survival if compared to creeks and streams occurring at lower altitudes.

The analysis of injured larvae frequencies revealed that, after six months of growing, more than a third of the total number of studied larvae had signs of injuries. As reported by Munshaw et al. (2014), the rate of injuries may reflect the level of intraspecific aggression. Density and likely starvation are factors that are supposed to enhance intraspecific aggression and cannibalism attempts in the fire salamander larvae (Reques & Tejedo 1996). In the tiger salamander, A. tigrinum, yearling larvae have increased injuries because of predation by older larvae, without morphological differences between cannibals/non-cannibals individuals (Wissinger et al. 2004). The rate of injuries may be considered a good proxy for intraspecific aggression in habitats where predators of amphibian larvae are missing (Munshaw et al. 2014). The rate found in our study was generally higher than that reported for caves and lower than that observed in cases of high predation pressure by Odonata larvae (Manenti et al. 2013). This fact indicates that larval density, that is higher in our pools than in caves, may enhance intraspecific aggression and confirms that in pools without predator occurrence the number of injuries is lower.
An interesting finding of this study is the observation of two abortive fire salamander eggs that were laid in October into the lowest pool (1160 m a.s.l.). Although observed only in the dissection of female uterus, abortive embryos were known to occur in one out of every five larvae of *S. s. salamandra* in central Europe and one out of every 20 larvae of *S. s. terrestris*, while for *S. s. fastuosa*, the number of abortive embryos may equal the number of viable larvae (Joly 1986). Their occurrence may be linked to a delay in embryos growth although in *S. s. fastuosa* it reflects an adap-
Development of the fire salamander larvae

Figure 4. The eggs with abortive embryos found in October 2013, with the newborn larvae. A = eggs and larvae together; B = close-up of an egg with the abortive embryo visible inside (photo by G. R).

tation to intra-uterus feeding (Joly 1986). In this subspecies, the viable embryos feed on the abortive eggs and few "giant" larvae, or already metamorphosed young, are laid by females after longer gestation periods (Joly 1986). Abortive embryos were always observed in dissected individuals, and before our finding, their laying in breeding waters was not reported in the literature. Together with the two eggs, we found eight new-born larvae of normal length in pool D. If fire salamander females have eggs with abortive embryos in their uterus, it may be likely that they lay them during larvae deposition, if the viable larvae did not consume them. More observations are necessary to understand if it has been an isolated event or if it happens more frequently in the studied pools.

Generally, our study provides new data on fire salamander larvae growing at high altitudes; growth, metamorphic timing, and age at metamorphosis of fire salamander larvae are affected by different environmental factors, such as temperature, availability of food, density of conspecifics and cohort effects (Kopp & Baur 2000, Denoël et al. 2001, Weitere et al. 2004, Eitam et al. 2005). Thus, further studies focusing on populations breeding in the mountain horizon could provide useful insights for understanding adaptive pressures involving the exploitation of such environments by this caudate in the Pre-alpine region.

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