

Cave exploitation by an usual epigeal species: a review on the current knowledge on fire salamander breeding in cave

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SUMMARY

The fire salamander (*Salamandra salamandra*) is a relatively common epigeal amphibian, widely distributed throughout Europe, which usually gives birth to aquatic larvae. Even if epigeal streams represent the most common places in which the species breeds, in some countries caves with underground waters are also used. To improve our understanding of the habitat features allowing successful breeding of salamanders in underground sites, we combined an exhaustive review of the available literature, especially the grey one, with direct observations performed from 2008 to 2017 in several natural and artificial caves of Lombardy, Liguria and Tuscany (Italy), Ariège and Provence (France). We provide a synthesis of published and unpublished caves in which the fire salamander breeding has been observed, along with a synthesis of the investigated ecological, behavioural and morphological traits. The use of underground sites is reported in several published papers and appears to be a common phenomenon not limited to single karst areas. The absence of predators, the relative stability of the aquatic habitats and the possibility to exploit new ecological resources are environmental factors that favour the breeding of the fire salamander. Our synthesis suggests that breeding of fire salamanders in caves is not a random event, but a widespread phenomenon that may be linked to specific biogeographical factors. Further insights may be obtained by performing genetic analyses on both cave and epigeal populations, and considering larger landscape scales for ecological studies as well. Gene flow between salamanders that breed in caves and in streams probably occurs, but on the other hand, assortative mating might limit it, thus allowing the conservation of local adaptations driving successful cave colonisation.

INTRODUCTION

Underground systems with groundwater play a fundamental role for humans as they represent a major source of potable water supply in many countries and, globally, the largest source of available freshwater (Culver and Pipan 2014). From the human perspective, since the prehistory, the interest for groundwater has focused on the operational management for potable or agricultural supply (Balland 1992). However underground environments such as caves and their aquifers, natural springs and draining galleries, can also be of particular interest from a zoological perspective (Stoch et al. 2009). Indeed, many subterranean aquatic habitats contain exceptional communities of organisms (Culver and Pipan 2009), which are receiving an increasing interest by zoologists as they can provide interesting ecological and evolutionary insights. Subterranean habitats have a high potential to permit the investigation of many aspects of modern ecology and biology (Pipan and Culver 2013). When analysing cave biota, it is important to recognize that underground habitats are not a closed system (Romero 2009): besides the exchange of abiotic components (e.g. chemicals, air and water) with outdoor environments, many organisms (more or less occasionally) move in and out caves, being entirely or for a part of their life cycles linked to underground environments (Lunghi et al. 2017).

Cave dwelling organisms are indicated with the prefix troglo- (meaning “cave” in ancient Greek) and, following the recent classification proposed by Sket (2008), they are divided in different categories. Generally obligate cave dwellers are named troglobionts, such as organisms that evolved specific adaptations to underground habitats, in which they spend their entire life cycle. Troglobionts often show peculiar morphological features, such as depigmentation and blindness. The biological relevance of these morphological adaptive features has been recently challenged by Romero (2011) who provided several cases of troglomorphic species being not associated to cave environment. Moreover, considerable morphological differences are frequently encountered among troglobiont animals, and some obligate cave dwellers even lack troglomorphic characters (Romero 2011).

Together with troglobionts, other three different categories of non-obligate cave dwellers are usually found in caves (Sket 2008). These

organisms are often associated with caves but do not necessarily show troglomorphic features. The categories are subtroglophiles, eutroglophiles and troglonexes. Subtroglophiles are “species inclined to perpetually inhabit a subterranean habitat” although remaining associated to the epigeous environment for some biological function; eutroglophiles are “essentially epigeous species” able to form permanent subterranean populations and troglonexes are considered as accidentals or stray organisms for cave environments (Sket 2008). Cave dwelling organisms inhabiting groundwater are indicated with the suffix “stygo” instead of “troglo” and the same categories of organisms may be found in the underground aquatic systems.

In many subterranean environments there is an enduring flux of organisms passively transported or actively migrating and invading. Recently, Lunghi et al. (2014), considering terrestrial communities of non-strict cave dwelling organisms, showed that different species usually thought to be exclusively epigeous did not occur randomly in caves. These species mostly select underground environments on the basis of their features, suggesting that their classification as troglonexes or accidentals may not be reliable for their true occurrence underground. A similar situation has also been described for the aquatic underground habitats, in which both organisms that could be defined as stygobionts and organisms that could be defined as stygophiles and stygoxenes respond, although differently, to the features of caves (Manenti 2014b). Distribution of stygobionts, stygophiles and stygoxenes appears to be not random, but linked to specific factors that differ between species (Manenti 2014b). Generally, Culver and Pipan (2014) report three different reasons for the active exploitation of cave habitats by species that are normally found outside. The first is the avoidance of environmental extremes that can occur in surface environment. This is the case for example of the populations of *Hydromantes* salamanders that during the dry seasons enter cave habitats (Salvidio *et al.* 1994). The second reason is the avoidance of competitors and predators that are usually much more frequent in epigeal rather than in hypogean habitats. This is, for example, the case of different species of collembolan that may find in caves a safer habitat than epigeal leaf litters (Kur *et al.* 2016). Finally, the colonisation of subterranean habitats may also represent for epigeal species the possibility to exploit new resources. In this case,

subterranean habitat furnishes the availability of major or different resources, the exploitation of which gives advantages to the individuals able to reach them; this situation has been demonstrated for different tropical caves but is also possible in temperate regions (Culver and Pipan 2014).

However, most of studies on underground animals focus on obligate cave dwellers, while only few consider underground populations of usually epigeous species (Lunghi et al. 2015; Lunghi et al. 2017).

Amphibians are among the vertebrates more often observed in caves. Amphibians occurrence in caves and in other hypogean habitats is often reported (Biswas 2014; Fenolio et al. 2005; Rosa and Penado 2013). The observation of amphibians in caves is particularly frequent for salamanders, and at least 11 species or subspecies of urodeles, belonging to the families Proteidae and Plethodontidae, are obligate cave-dwelling organisms showing troglomorphism (Romero 2009). Moreover, many species during their life cycle are more or less regularly linked to cave environments and can play important roles for cave ecosystems (Lavoie et al. 2007).

For instance, the Pyrenean brook salamander (*Calotriton asper*) usually lives in epigeal streams, but shows also cave-dwelling populations which complete their entire lifecycle underground and are strongly isolated from the epigeal populations (Clergue-Gazeau 1975; Dreiss et al. 2009; Issartel et al. 2010; Uiblein et al. 1992). Concerning the Italian herpetofauna, nine species of amphibians live in strong association with underground environments (the troglomorphic olm *Proteus anguinus* and the Plethodontid cave salamanders belonging to the genus *Hydromantes*), but at least 17 additional species have been more or less occasionally found in underground spaces (Bologna 1982; Bressi and Dolce 1999). However, breeding in subterranean environments has been observed for a limited number of them: the Alpine newt *Mesotriton alpestris* (Melega and Fusini 2000), the spectacled salamander *Salamandrina perspicillata* (Angelini and Cari 2004; Razzetti et al. 2001), the common toad *Bufo bufo* (Bonini et al. 1999), the common frog *Rana temporaria* (Bressi and Dolce 1999) and the fire salamander *Salamandra salamandra* (Bressi and Dolce 1999; Gasparo 2002; Manenti 2008, 2009; Razzetti et al. 2001; Uhrin and Lesinsky 1997).

The fire salamander is usually an epigeous species breeding in streams (Manenti et al. 2009b), but in several areas, this species has been regularly found in caves and in others underground habitats. Even if in some cases caves can act as traps for both adults and larvae (Uhrin and Lesinsky 1997), there is a number of active mechanisms that can explain the underground occurrence of salamanders. Subterranean habitats can be used as winter shelters, hiding places during the active season, feeding habitats (Balogova and Uhrin 2014) and also as regular breeding sites (Manenti et al. 2009a). Although several studies documented the presence of amphibians in caves, investigations on the factors determining amphibian distribution and reproduction in underground environments remain scarce. Amphibian species that are not obligate cave dwellers, but for which some populations live in caves, can provide useful case studies to understand factors allowing the colonization of strongly different habitat, and the relative importance of local adaptations and phenotypic plasticity for the exploitation of novel habitats.

The aim of this paper was to review and update the current knowledge of the exploitation of underground environments by fire salamanders in the Alps and Apennines. We combined published and unpublished data on salamander breeding in underground habitats, to improve our understanding of the habitat features allowing successful breeding of salamanders in underground sites.

MATERIALS AND METHODS

Observations of fire salamander in caves and factors allowing breeding

To summarize the actual knowledge about cave breeding by the fire salamander (*Salamandra salamandra*), we combined an exhaustive review of the available literature, especially the grey one, with direct observations performed from 2008 to 2017 in several natural and artificial caves of Lombardy, Liguria and Tuscany. To obtain preliminary information of caves (i.e. location, development) we used the data from the Lombardian, Ligurian and Tuscan Speleologic Register. As artificial underground sites, we considered subterranean artificial springs as draining galleries of catchment (the so called 'bottini'), and artificial mines. To localize the artificial sites, we used information available in studies on stygophiles and stygobious mollusks (Pezzoli 1996, 2010) and local information on mine features. We performed the

surveys from February to July exploring the caves as deeply as possible and looking for larvae of *S. salamandra*. In each site fire salamander larvae occurrence was assessed by multiple surveys both during day and night. Additionally, in May 2009 and July 2016 some surveys were performed on 15 artificial subterranean sites of Provence and Ariège (France).

From 2008 to 2010, from February to April, we characterized the environmental features determining fire salamander breeding in a subset of 61 natural caves and 30 artificial underground springs. For each site we recorded different environmental variables that were related to larvae presence/absence: maximum illuminance occurring at 6 m from the entrance (or at the end of the cave in shorter sites) using a CEM DT8820 multiparameter (range 0.01–200,000 lux), altitude, slope of the territory outside the cave above and below the entrance in a radius of 15 m; presence of water inside the cave; distance of water from the entrance; total extension of the cave; richness of the community of benthonic macro-invertebrates, measured as the number of taxonomic units (see (Ghetti 1997). Macrobenthos was collected by moving the substrate for 10 minutes with a thin-mesh dip net (mesh size: 1 mm).

Field surveys on larvae development, ecology and behaviour

From 2010 we performed field observations on 30 populations breeding in both artificial and natural caves of the NW Italy observing the larval development in underground conditions, assessing factors related to larvae abundance and activity, and evaluating the differences between biomass balance at fire salamander larvae level in caves and in nearby epigeal sites.

In each site, all observed larvae were captured using a small dip net. Catching lasted for at least 10 minutes after the last captured larva. We assessed total larvae number using two successive removal samplings. Larvae kept in the first sampling were maintained in a small aquarium (20×10 cm, depth 5 cm), and the capture session was repeated 30 min later. Larvae detectability is generally high in pools (Manenti et al. 2009b) and the removal method proposed accounts for the sampling effort caused by the different environmental conditions of the pools (Schmidt et al. 2015). Density estimation was obtained applying the removal method proposed by Chao and Chang (1999). For each

larva, we recorded total length (accuracy: 1 mm) and weight (accuracy: 0.01 g). Furthermore, larvae were photographed laterally (Eitam and Blaustein 2002) to allow individual identification and follow development and growth.

In a subset of 21 fire salamander breeding sites (12 cave pools and 9 spring pools) we weekly monitored during day time from March to August 2013, and from March to August 2014, the larvae deposition, development and metamorphosis. In these 21 sites, on the basis of individual recognition, we evaluated the mortality rate of each site on the basis of the number of recaptured salamanders: we considered as "dead" all the larvae that were recorded at early stages at a certain survey and that were never recaptured in the successive surveys.

In 52 distinct sites (15 cave pools and 37 epigeal breeding sites like springs and stream pools), during both day and night we estimated the number of active fire salamander larvae, recording the number of larvae visible from the pool shore in 5 minutes of survey. The active larvae were divided in central and external, depending on their position. Every pool was divided in internal and external contiguous sectors of approximately the same area. To calculate the internal and external areas, before larvae counting, we positioned 2 metric strings along the pool maximum width and length and we considered as internal the central area formed by approximately 2/3 of the pool maximum width and length. We quickly numbered larvae on the basis of their position doing also pictures of the whole pool (using infrared camera for night or cave conditions). We then calculated the frequency of active larvae as a proportion of the total number of collected larvae. As environmental variables, we recorded both abiotic and biotic variables. During each survey and for each pool, we recorded surface area on the basis of pool maximum width and length, we measured the maximum water level and recorded water temperature measured in the middle of the pool at 2 cm from the substrate during the same daytime range (10–12 a.m.). We also recorded the maximum illuminance incident on the pool surface using a CEM DT8820 multiparameter (range 0.01–200,000 lux). As biotic parameters we considered the predation pressure (measured as the occurrence and biomass of predators), intraspecific competition (abundance of salamander larvae) and an estimate of prey availability (biomass of potential prey). We used Anova with Tukey post-hoc test to disentangle

the interactions between day/night and cave/epigeal activity.

To assess microhabitat features determining larvae distribution inside caves we performed repeated surveys during every season of 2013. We surveyed all the 15 caves with pools (for each cave minimum 4, maximum 6 and average 4.5 surveys) within the upper Cosia Valley (Como district), a karst area in Lombardy (NW Italy). All the 31 pools were sampled and characterized recording: a) distance from cave entrance b), maximum received illuminance, c) pool width, d) pool maximum depth, e) maximum depth range reached by the pool during the surveys, measured as the difference between the maximum and the minimum depth recorded, f) pool average temperature and g) maximum temperature range reached by the pool during the surveys, measured as the difference between the maximum and the minimum temperature recorded. We finally assessed aquatic invertebrates occurrence performing in each pool 3 minutes of visual encounter survey followed by 7 minutes of substrate sampling using a thin-mesh dip net; we distinguished between invertebrates with at least one troglomorphic character like depigmentation, blindness or appendages elongation (e.g. *Niphargus ambulator*, *Dendrocoelum* n. sp.) from those without any troglomorphic character (e. g. molluscs, hirudineans, plecopterans, trichopterans and coleopterans).

RESULTS AND DISCUSSION

Observations of fire salamander in cave and factors allowing breeding

Cave investigations allowed us to find 219 natural caves and 73 artificial underground sites in Lombardy, Liguria and Tuscany (Italy); within all surveyed sites, 123 hosted subterranean pools or streams. We recorded 52 underground breeding sites of the fire salamander: 15 occurred within natural caves, while 37 were artificial sites like draining galleries, “bottini”, mines and abandoned tunnels (Table 1). The surveys performed in France allowed us to record fire salamander breeding in artificial mines of the Pyrenees. The surveys performed in Provence were unfruitful as all the draining galleries surveyed were of limited access for amphibians because normally closed by doors (whose opening is regulated and generally rare) that cover all the entrance surface. Overall, considering also the data from the literature and the single site that we found in the French Pyrenees, we obtained

77 observations of fire salamander breeding in caves (Appendix I). Breeding in caves has been recorded across the whole range of *Salamandra salamandra*, and occurs in both natural and artificial caves. We recorded observations published for eight European countries. Some other observations of breeding have been recorded for eastern Liguria in artificial mines (C. Pia, personal observation). Cases of breeding in underground sites have also been recently discovered in Switzerland (P. Scimè, personal communication) and are reported for several subterranean habitats of Germany (Schiemenz and Günther 1994; Uthleb et al. in press). Malkmus and Zaenker (2008) found larvae in 4.8 % of the artificial cavities, and in one natural cave over 1275 subterranean sites investigated in Hessen (Germany). In the Harz Mountains, almost all of the galleries with water bodies were used for breeding (Arnold 1986; Uthleb et al. in press), as well as in West Saxony, where larvae were repeatedly found in mines (Arnold 1978; Büttner 1926), and in Thuringia too (Uthleb et al. in press).

In appendix I we included only observations in which the active deposition of larvae has been clearly stated and was linked to a certain site. Globally, the 27% of the reported caves are natural sites, while the remnant refers to different typologies of artificial subterranean habitats (Appendix I). The breeding of fire salamander larvae is reported not only in artificial structures built to catch water, like draining galleries but also in mines and other gallery typologies. The general distance of larvae occurrence from the entrance, considering both the data of our surveys and the observations found in the literature, varies from 0 to 170 m. The results from the subset of caves in which we performed environmental characterization revealed that fire salamander chose underground sites with particular features.

Ecological determinants of cave use for breeding may be inferred by our analyses performed on the subset of artificial and natural investigated caves. A first survey performed on artificial springs with hypogean development showed that the fire salamander selects draining galleries with stable water presence (Manenti 2008). Subsequent, more detailed analyses underlined that the species breeds in underground springs with high ease access and with relatively rich macrobenthos communities (Manenti et al. 2009a). The surveys were successively extended to natural caves and compared to the already known artificial

underground springs. Breeding occurred in the most accessible caves, with gentle slope below the entrance, with pools nearby the entrance and hosting rich macrobenthos communities (Manenti et al. 2011). Occupied natural and artificial caves had

similar environmental features. The analyses performed confirmed the fire salamander ability to breed in underground damp biotopes if placed in a suitable environmental context.

Table 1. Results of cave surveys performed from 2008 to 2017 in Italy.

Region	Sites explored	Natural caves	Artificial caves	<i>S. salamandra</i> breeding sites	Natural caves with breeding	Artificial caves with breeding
Lombardy	132	82	50	44	13	31
Liguria	88	78	10	7	2	5
Tuscany	72	59	13	1	0	1
Total	292	219	73	52	15	37

Effect of cave environment on larvae development, published studies

We performed surveys on larval development, in both underground springs and natural caves, comparing the larval growth between epigeal and underground environments. In underground springs, larvae showed a longer development and metamorphosis occurs at larger body size when compared to the larvae from nearby epigeal streams (Manenti et al. 2009b; Manenti et al. 2011). Using capture-mark-recapture, we tracked individuals along their whole development, analysing the factors affecting larval growth and comparing individuals living in underground springs with those from the epigeal ones. Growth rate was faster in environments with abundant invertebrates and few conspecific larvae; moreover, there were clear differences between habitats. Larval salamanders generally feed on benthic macroinvertebrates. However, the growth rate of salamander larvae was positively related to the abundance of macrobenthos in streams, but not in caves. Macrobenthos abundance was extremely low in caves, and it is possible that in this environment larvae have to change their diet, shifting from the predation of macrobenthos to different strategies, such as the predation of ostracods or copepods crustaceans smaller than 0.55 mm, the consumption of organic detritus or dead insects, and cannibalism as hypothesised by Limongi et al. (2015).

Effect of cave environment on larvae mortality, original results

During larval development, cave habitat was the only significant factor affecting mortality rate as evidenced by an analysis with Linear Models (Table 2). In caves, predator occurrence was extremely rare (Manenti et al. 2013b). The only detected predators during all the surveys was the native freshwater crayfish *Austropotamobius pallipes*, found in a draining gallery, and the dragonfly larvae of the species *Cordulegaster bidentata*, observed in two sites. In epigeal sites we detected predators in 36% of the weekly surveyed pools. Predators comprised the freshwater crayfish *A. pallipes* and dragonfly larvae of the genera *Cordulegaster* and *Aeshna*.

Larvae activity in caves published and original results

The activity of fire salamander larvae was related to the risk of being preyed and surface streams showed a higher predation risk than caves (Manenti et al. 2016). We observed strong differences on the rate of active larvae both between subterranean water and epigeal spring pools, as well as during day or night time. There were significantly more larvae active during night ($F = 31.47, P < 0.001$) and in caves ($F = 19.35, P < 0.001$). In caves the rate of active larvae was similar between night and day ($P = 0.77$), while in epigeal springs it was quite lower during the day ($P < 0.001$). No significant interactions occurred between day/night activity and predators occurrence. However, the rate of active larvae was significantly lower when predators biomass was high ($F = 6.22, P = 0.01$). Also the density of prey ($F = 6.5, P = 0.01$) and the pool illuminance ($F = 4.5, P < 0.03$) affected fire

salamander activity. There were more active larvae when prey were abundant and light was lower. Considering the space use by the active larvae, the frequency of larvae in lateral sectors was negatively influenced by the biomass of aquatic predators ($B = -0.21$, $F = 5.35$, $P = 0.02$). The rate of larvae in central sectors was positively linked to cave habitat ($B = 0.05$, $F = 7.9$, $P < 0.01$) and negatively to illuminance ($B = -0.02$, $F = 6.8$, $P = 0.01$).

Table 2. Results of the Linear Models analysis of the relationship between fire salamander larvae, mortality rate and the environmental features in 21 spring and cave sites.

Variable	NumDf	DenDf	B	F	P
Cave	1	15	0.13	71.9	0.01
Conspecifics density	1	15	-0.03	0.43	0.52
Prey biomass	1	15	0.19	13.92	0.26
Predators occurrence	1	15	0.02	0.01	0.98
Temperature	1	15	-0.6	0.9	0.36
Illuminance	1	15	-0.01	0.01	0.97

Table 3. Number of Italian underground sites in which fire salamander breeding occurred; data represent combination between field surveys and an extensive literature review.

Region	Total sites	Natural caves	Artificial caves
Lombardy	44	13	31
Liguria	7	2	5
Tuscany	1	0	1
Piedmont	4	0	4
Veneto	1	1	0
Friuli	3	3	0
Total	60	19	41

Subterranean microhabitat features of caves used for breeding: preliminary original results

Considering the effect of microhabitat features of pools inside caves toward the whole community of aquatic organisms found in all the caves of the upper Cosia Valley (Como district, Italy), a RDA analysis revealed that fire salamander larvae mainly occurred in smaller pools closer to the entrance and with higher average temperature excursion during the year (Manenti 2014b). An analysis performed

with linear mixed models considering the relationship between larvae occurrence and troglomorphic and non-troglomorphic taxa showed that the larvae are negatively related to the occurrence of troglomorphic species ($F = 5.97$, $P = 0.02$).

General considerations on cave exploitations by the fire salamander

Overall, the analysis of data from field surveys shows that some cave features, such as the stability of habitat (water permanence), the absence of predators and the availability of resources (Culver and Pipan 2014), may favour the exploitation of underground environments by a usually epigeal species like the fire salamander. Caves may furnish breeding habitats that are more stable, with a more regular hydroperiod, than epigeal streams and creeks, that especially in karst areas may be subjected to strong variation depending on the amount of rainfalls (Barzaghi *et al.* 2017; Culver and Pipan 2014). Moreover, cave pools are usually predator deprived habitats for the fire salamander larvae (Manenti *et al.* 2016) that occupy the top level of the aquatic food web in these habitats (Barzaghi *et al.* 2017). This aspect may also be connected with the possibility of caves to provide superior resources than epigeal habitats (Culver and Pipan 2014). The absence of other predator could represent for fire salamander the absence of competitor for the invertebrate prey occurring in these habitats. However, the previous studies and our observations underline that prey is generally scarce in these habitats (Culver and Pipan 2009; Fenolio *et al.* 2006). The cave habitat thus poses also different trophic constraints to the fire salamander larvae, as we will detail here below.

Generally, our review underlines that the use of caves is not limited to small geographical areas but seems to be quite frequent across the whole fire salamander range (Table 3). The study of cave use for reproduction by a usually epigeal species may provide interesting insights from a biogeographical point of view. Different experimental studies have been performed on the role played by local adaptations and phenotypic plasticity in allowing cave exploitation by the fire salamander. Some preliminary laboratory experiments on the effect of underground conditions on fire salamander larvae date back to the 1980's. Gimenez-Lopez and Guarner Deu (1982) collected larvae in an epigeal spring and reared them

underground in a natural well, to observe the effect of cave environment on their development. The strong impact of underground conditions on larval growth was already evident in this study, as larvae required 22 weeks to metamorphose while their siblings took 7-10 weeks in normal outdoor conditions. In amphibians, the time required for metamorphosis in hypogean habitats, is strongly affected by environmental features such as temperature and food availability: development rate is generally slower in cold conditions and with scarce food, and these factors likely explain the longer time required for metamorphosis. However, beside the cold temperature and the scarce food, many more environmental features differ between underground and outdoor sites. For instance, salamanders are active predators, and the lack of light can strongly influence prey detection and predation behaviour. Recent studies showed that in complete darkness, both cave-born and stream-born larvae are able to detect and capture prey, but larvae from caves capture prey with higher success than larvae from stream populations, suggesting that cave populations evolved local adaptations improving predation performance in underground environments (Manenti and Ficetola 2013). Moreover, larvae from all the populations show a plastic behaviour, as under light conditions they adopt a sit-and-wait predation strategy, while in complete darkness they perform an active search of prey, during which they continuously explore the environment and move longer distances (Manenti et al. 2013a). However, the plastic response was not constant among populations, as cave born larvae showed higher behavioural plasticity than stream larvae, and better exploited the available space in test environments.

Salamanders in caves face major challenges, such as food scarcity (Limongi et al. 2015). In all streams, springs and caves, it is frequent to find larvae with different ages showing strong asymmetries in terms of both size and developmental stage (Manenti et al. 2011; Romeo et al. 2015). Larvae from both caves and streams often display aggressive behaviours, and the frequency of intraspecific attacks increases with starvation and larvae density (Manenti et al. 2015). The fact that hungry larvae display more aggressive behaviours suggests that the internal status of the starved larvae affects the apparent resource value of the conspecifics: hungry larvae would score a food resource more highly than would a satiated one

(Arnott and Elwood 2008). Smaller fire salamander larvae may represent an important food resource and may be easily attacked by the largest ones. Indeed, the occurrence of full grade cannibalism has been often reported, especially in pools or stream where larvae density was high (Joly 1968). In food deprived habitats, cannibalism can play an important role for metamorphosis achievement, allowing the first cohorts of larvae to feed on later-arriving cohorts and reach metamorphosis as shown in the congeneric *Salamandra infraimmaculata* (Markman et al. 2009). This species breeds in temporary ponds with poor trophic conditions. In *S. infraimmaculata* larvae, trophic function is linked by their timing of deposition. In temporary pools, while all the larvae compete for the limited prey resources, the first cohorts cannibalize later-arrived ones (Sadeh et al. 2009; Sadeh et al. 2011; Segev et al. 2010). When there are no yet other larvae, *S. infraimmaculata* gravid females opt for deposition in structurally simple habitats, but if conspecific larvae already occur, they prefer more complex habitats; habitat complexity favour sheltering and larval survival in cannibalistic populations (Sadeh et al. 2009). Similar mechanisms may act in environments with limited resources, such as caves, where starvation periods may be frequent and prolonged. Studies made on larvae from caves and from neighbouring streams showed that, while fire salamander larvae are scarcely aggressive in early developmental stages, their aggressiveness quickly increases with the age (Manenti et al. 2015). Again, this behavioural change is not the same in all the populations, as aggressiveness increased with age more quickly in larvae from caves than in those from streams. Starvation condition, high conspecifics density and competition are the factors much more conditioning aggressiveness (Manenti et al. 2015).

CONCLUSIONS

Overall the current knowledge on fire salamander breeding in caves, stresses that local adaptations and phenotypic plasticity act in concert during the colonization of caves, highlighting that a strong phenotypic plasticity is a key trait for survival in these extreme environments.

Globally the variation in the different aspects that were till now studied was strong and involved complex interactions between plasticity and local adaptations. Larvae from cave populations showed higher plasticity than stream larvae for

several behavioural features, such as predation behaviour and aggressiveness, underlining the importance of plasticity in allowing the exploitation of resource-poor environments like caves. No genetic data are until now available. As reported above, fire salamander females typically deposit their larvae in small first-order streams, but other than caves in some cases they can use small ephemeral ponds for larval deposition (Denoël and Winandy 2014; Weitere et al. 2004). Specific studies performed in the Kottenforst, a deciduous forest in western Germany, revealed the existence of two sympatrically emerging genetic lineages, well matching the adaptation to the different larval freshwater habitats, such as permanent streams and ephemeral ponds (Steinfartz et al. 2007). The mechanisms explaining this differentiation can include assortative mating and habitat choice by females (Caspers et al. 2015; Steinfartz et al. 2007). However, given that fire salamanders are quite mobile, (Ficetola 2012; Schmidt et al. 2007; Schulte et al. 2007) and caves and streams often occur within the dispersion capability of a single population, gene flow probably occurs between salamanders that breed in caves and in streams. Once metamorphosed, salamanders are able to exit caves and mate with other salamander that are born in the surrounding epigeal streams. On the other hand, at least in the most isolated sites, assortative mating might limit the gene flow between populations breeding inside and outside caves. In that case, isolation could allow the conservation of local adaptations that are driving successful cave

exploitation. However, further research is needed to assess the genetic differences between larvae occurring in caves and in the nearby streams, to obtain genetic measures of local adaptations, and to identify the processes allowing the persistence of local adaptations over such fine spatial scales. In the last years, approaches based on genome scan have provided valuable information on local adaptations of non-model species in natural environments (Catchen et al. 2017). Unfortunately, most of urodeles have very large genomes (>40 gigabases) (Mueller et al. 2008), and this makes challenging the use of population genetic tools such as the Restriction site-associated DNA Sequencing (RAD) sequencing. This is a cheap and efficient method for genotyping that samples a shared set of sites across the genome in numerous individuals or pools (Davey et al. 2013), but with the large genomes of urodeles its efficiency may be reduced. However, in the next future approaches based on transcriptomics might allow a fine-scale analysis of variation of populations, and helping to identify the genetic processes allowing the successful colonization of underground environments. Moreover, as the observations reported in this paper underline that the use of caves is quite widespread and not limited to single areas, further approaches may involve behavioural and evolutionary experiments to be performed at larger landscape scales. In particular, to explain biogeographical factors determining fire salamander distribution in artificial and natural caves, populations from different karst and non-karst areas could be compared.

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Appendix I. New and published records of fire salamander breeding in caves or other underground habitats.

Nation	Region	Locality	Typology	spatial development	Distance from entrance	Reference
France	Alsace	Mine	Mine	-	50	(Baumgart 1981)
France	Alsace	Mine	Mine	-	150	(Baumgart 1981)
France	Alsace	Mine "P"	Mine	-	14	(Baumgart 1981)
France	Ariège	Mine de Seix	Mine	40	0	this study
France	Bourgogne	Carrière de Gypse,	Mine	-	-	(Roué 2012)
France	Bourgogne	Ignota	Mine	-	-	(Roué 2012)
France	Bourgogne	Ruisseau	Mine	-	-	(Roué 2012)
Italy	Friuli	Bus de le Fade	Natural cave	-	10	(Bressi and Dolce 1999)
Italy	Friuli	Ciase de Lis Aganis	Natural cave	554,00	70	(Bressi and Dolce 1999)
Italy	Friuli	Grotta II di La Val	Natural cave	1750,00	15	(Bressi and Dolce 1999)
Italy	Liguria	Miniera di Maissana	Mine	20	3	this study
Italy	Liguria	Monte Caucaso	Mine	30	19	this study
Italy	Liguria	Monte Caucaso	Mine	50	10	this study
Italy	Liguria	Monte Caucaso	Mine	100	15	this study
Italy	Liguria	Pertuzo Do Paolin	Natural cave	15	1	this study
Italy	Liguria	San Bartolomeo	Artificial cave	-	10	(Razzetti et al. 2001)
Italy	Liguria	Tanna da Suja	Natural cave	300	30	this study
Italy	Lombardy	Blessagno	Bottino		1	This study
Italy	Lombardy	Pellio	Bottino		2	This study
Italy	Lombardy	Boeucc di La Val	Natural cave	15	2	(Manenti et al. 2010)
Italy	Lombardy	Bottino I di Val Calolden	Bottino	1,5	0	this study
Italy	Lombardy	Bottino II di Val Calolden	Natural cave	2,5	0	this study
Italy	Lombardy	Briosco, Cascina Madonnina	Draining gallery	4,10	0	(Manenti and Bianchi 2010)
Italy	Lombardy	Bulciago	Draining gallery	3,74	0	(Manenti 2008)
Italy	Lombardy	Canzo, second'alpe	Bottino		1	(Manenti et al. 2009a)
Italy	Lombardy	Canzo, second'alpe	Bottino		1	(Manenti et al. 2009a)
Italy	Lombardy	Canzo, second'alpe	Bottino		1	(Manenti et al. 2009a)
Italy	Lombardy	Cassago, Zizzanorre	Bottino		1	(Manenti et al. 2009a)
Italy	Lombardy	Castello di Brianza Val Sorda	Draining gallery	8,70	1	(Manenti 2008)
Italy	Lombardy	Castello di Brianza Val Sorda	Bottino		1	(Manenti et al. 2009a)
Italy	Lombardy	Tomba del Polacco	Natural cave	2000	10	this study
Italy	Lombardy	Cernobbio, Scala Santa	Bottino	6	3	this study
Italy	Lombardy	Colle Brianza, Campanone	Bottino			this study
Italy	Lombardy	Dolzago, Prato d'Hoè	Bottino		0	this study
Italy	Lombardy	Ello	Bottino		0	(Manenti et al. 2009a)
Italy	Lombardy	Ello	Bottino		1	(Manenti et al. 2009a)
Italy	Lombardy	Ello, Fura	Bottino		0	(Manenti et al. 2009a)

Italy	Lombardy	Eremo	Draining gallery	50,00	30	(Manenti 2014a)
Italy	Lombardy	Esino Lario, Ortanella	Bottino		1	Manenti et al 2009
Italy	Lombardy	Fonte della Mojenca	Draining gallery	18,00	0	(Manenti 2014a)
Italy	Lombardy	Grotta del Mocc	Natural cave	25	12	(Manenti et al. 2010)
Italy	Lombardy	Grotta della Corna di Bobbio	Natural cave	15	3	(Manenti et al. 2010)
Italy	Lombardy	Grottina del ceppo	Draining gallery	11,40	1	(Manenti 2014a)
Italy	Lombardy	Inverigo Pampello	Bottino		1	this study
Italy	Lombardy	Inverigo, Becogne	Bottino		0,5	this study
Italy	Lombardy	Inverigo, Orrido	Bottino		0	(Manenti et al. 2013b)
Italy	Lombardy	Inverigo, Orrido	Bottino		0	this study
Italy	Lombardy	La Burlunga	Natural cave	30	0	(Manenti et al. 2010)
Italy	Lombardy	La Val	Natural cave	6	2	(Manenti et al. 2010)
Italy	Lombardy	Lego	Natural cave	150	50	(Manenti et al. 2010)
Italy	Lombardy	Miniera di Valbondione	Mine	20	3	this study
Italy	Lombardy	Miniera Tracciolino	Railway gallery	340	170	this study
Italy	Lombardy	Posca sorgente 1	Draining gallery	70,00	1	(Manenti and Bianchi 2010)
Italy	Lombardy	Posca sorgente 2	Bottino	3,00	1	(Manenti and Bianchi 2010)
Italy	Lombardy	Sorgente 13 PMB	Draining gallery	6,97	1	(Manenti and Bianchi 2010)
Italy	Lombardy	Sorgente Salamandra	Natural cave	9	2,5	(Manenti et al. 2010)
Italy	Lombardy	Sorgente sotto il Parcheggio	Natural cave	29	0	(Manenti et al. 2010)
Italy	Lombardy	Tana della Bavaresa	Natural cave	10	4	(Manenti et al. 2010)
Italy	Lombardy	Tana della Volpe	Natural cave	2600	10	This study
Italy	Lombardy	Tanetta	Natural cave	10	5	(Manenti et al. 2010)
Italy	Lombardy	Valmadrera, Sambrosera	Bottino		0	(Manenti et al. 2009a)
Italy	Piedmont	Laghi della Lavagnina	Mine		50	(Razzetti et al. 2001)
Italy	Piedmont	Laghi della Lavagnina	Mine		50	(Razzetti et al. 2001)
Italy	Piedmont	Laghi della Lavagnina	Mine		50	(Razzetti et al. 2001)
Italy	Piedmont	Miniera di Fontane	Mine		20	(Razzetti et al. 2001)
Italy	Tuscany	Miniera di Calcaferro	Mine	30	6	this study
Italy	Veneto	Grotta di Sant'Antonio	Natural cave	33	30	(Gasparo 2002)
Portugal	Central -North	Serra da Estrela	Draining gallery	25	3	(Rosa and Penado 2013)
Romania	Padurea Craiului Mountains	Valau cave	Natural cave	-	10	(Ianc et al. 2012)
Slovakia	Spiš-Gemer Karst	Bobačka	Natural cave	3036	1	(Balogova et al. 2017)
Slovakia	Spiš-Gemer Karst	Tichá Voda	Draining gallery	350	20	(Balogova et al. 2017)
Slovenia	Kras	Galleria d'acqua Vroce	Draining gallery	-	20	(Bressi and Dolce 1999)
Spain	Ciudad Real	Valley of the River Ojailén	Road tunnel	-	-	(Herrero and Hinckley 2014)
Spain	Ciudad Real	Valley of the River Ojailén	Road tunnel	-	-	(Herrero and Hinckley 2014)
Spain	Ciudad Real	Valley of the River Ojailén	Road tunnel	-	-	(Herrero and Hinckley 2014)

Spain	Ciudad Real	Valley of the River Ojailén	Road tunnel	-	-	(Herrero and Hinckley 2014)
Spain	Ciudad Real	Valley of the River Ojailén	Road tunnel	-	-	(Herrero and Hinckley 2014)