

Is the Italian stream frog (*Rana italica* Dubois, 1987) an opportunistic exploiter of cave twilight zone?

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Abstract

Studies on frogs exploiting subterranean environments are extremely scarce, as these Amphibians are usually considered accidental in these environments. However, according to recent studies, some anurans actively select subterranean environments on the basis of specific environmental features, and thus are able to inhabit these environments throughout the year. We present the first study on the abundance and spatial use of the Italian stream frog, *Rana italica*, in subterranean environments. We monthly collected data from 66 cave sectors during a whole year (2013), recording > 120 detections of *R. italica*. Frogs were more frequently found close to the cave entrance, without significant differences between age classes or sexes. Adults generally were observed being higher up along cave walls compared to juveniles. Frogs abundance was higher in areas showing specific environmental features, such as warm temperature, low incident light and the presence of potential prey. *Rana italica* likely occupies subterranean areas characterized by a combination of microclimatic suitability and prey availability.

Keywords

biospeleology, Anuran, amphibian, cave biology, prey, microclimate, spatial use, food web

Introduction

Several amphibian species are known to regularly exploit subterranean environments but, besides numerous reports concerning several salamanders species (Niemiller and Miller 2007, Pierce et al. 2014, Manenti et al. 2017, Soares et al. 2017, Vörös et al. 2017), only a few species of frogs and toads (Anura) are known to exploit these environments (Fenolio et al. 2005, Köhler et al. 2010, Lunghi et al. 2014, Koller 2017). Frogs have been usually considered as an accidental presence in subterranean environments (Bressi and Dolce 1999), but recently many authors showed that some Anuran species can be quite common in these sites, selecting caves with specific environmental features (Köhler et al. 2010, Rosa and Penado 2013, Biswas 2014, Lunghi et al. 2014, Matavelli et al. 2015). One of these species is the Italian stream frog *Rana italica* Dubois, 1987, which is endemic of Italian Apennines (Lanza et al. 2006, Canestrelli et al. 2008, Buono et al. 2014, Sindaco and Grieco 2014). According to the known habitat requirements, *R. italica* is strongly bounded to freshwater environments and usually occurs in forested areas; in some circumstances, the species is also found in natural and artificial subterranean sites, where it seems to be able to breed (Lanza et al. 2006, Vanni and Nistri 2006). Although most of the previous observations of *R. italica* refer to individuals trapped in wells and vertical caves (Bressi and Dolce 1999), recent studies underlined that this frog is able to actively exploit subterranean environments during the whole year (Lunghi et al. 2017).

Subterranean environments are characterized by peculiar microclimatic features: in temperate areas, they generally have a relatively constant temperature, which roughly corresponds to the mean annual temperature at the surface. Furthermore, air humidity is generally very high, and incoming light is only present within the first meters after the cave entrance (Romero 2009, Lunghi et al. 2015). Close to the surface (cave entrance and twilight zone), the inner microclimate is mostly affected by external environmental conditions, and such peculiarity can promote the abundance of troglophile species (Manenti et al. 2015, Lunghi et al. 2017). The microclimate characterizing the shallow part of subterranean environments seems to be particularly suitable for *Rana italica* (Lunghi et al. 2014); therefore, the use of subterranean spaces during periods characterized by unsuitable (e.g., dry) surface conditions may be of key importance for population survival (Forrester et al. 2015, Lunghi et al. 2017). Beside a suitable microclimate, in subterranean environments *R. italica* probably finds shelter from predators (Vanni and Nistri 2006). Nevertheless, information on the biology of Italian stream frogs in subterranean environments, and on the relevance of these habitats for its conservation, is extremely limited. With this study we aim to provide the first assessment of *R. italica* in subterranean environments, mostly focusing on population abundance and habitat use.

Methods

Data collection

From January 2013 to December 2013, we monthly surveyed nine subterranean environments located in the north of Tuscan Apennines (between 43°53'17"N, 11°06'22"E and 44°03'48"N, 10°48'09"E), where the presence of *Rana italica* was already assessed by a previous study (Lunghi et al. 2014). All surveyed environments did not show a morphology hampering frogs to move freely between subterranean and outdoor areas. Three of them were World War II refuges, one was a drainage tunnel, while the remaining six were natural caves (Table 1). Subterranean environments were explored until the point in which speleological equipment was necessary, and only when environmental conditions did not pose a risk (e.g., flooding). All studied environments were divided into portions of 3-linear meters of length (hereafter sectors); this subdivision allows a good data collection for both biotic and abiotic features characterizing subterranean environments (Lunghi et al. 2015, Manenti et al. 2015, Lunghi et al. 2017). Within each sector we recorded air temperature and humidity (measured with a Lafayette TDP92 thermo-hygrometer; accuracy: 0.1 °C and 0.1%) and the average incident light (obtained by averaging max and min illuminance recorded with a Velleman DVM1300 light meter; minimum recordable light: 0.1 lux). We recorded the abundance of *Rana italica* within sectors using visual encounter surveys (VES; Crump and Scott 1994) and adopting a standardized survey method (7.5 min/sector) which limits potential effects of imperfect species detection (Banks-Leite et al. 2014, Lunghi et al. 2017). Using the same procedure (VES + standardized survey method), we assessed the presence of seven invertebrate species which potentially represent prey items for *R. italica*: one dipteran (*Limonia nubeculosa* Meigen, 1804), three spiders (*Meta menardi* (Latreille, 1804), *Tegenaria* Latreille, 1804 sp., *Mettellina merianae* (Scopoli, 1763)), one cricket (*Dolichopoda laetitiae* Minozzi, 1920) and two gastropods (*Chilostoma planospira* (Férussac, 1832), *Oxychilus draparnaudi* (Beck, 1837)). All considered species have stable populations in subterranean environments, even if they are not obligate cave species (Lunghi et al. 2017). We measured the snout-vent length (SVL) of all captured frogs (Fig. 1a); individuals with SVL \leq 30 mm were considered juveniles (Buono et al. 2014). Within adults, we recognized males on the basis of secondary sexual characters (nuptial pads). For each frog, we measured the distance from the cave entrance and the elevation from the cave ground floor.

Statistical analyses

We used Generalized Linear Mixed Models (GLMM) to assess whether abundance of *Rana italica* was related to both biotic and abiotic recorded parameters. As dependent variable we used the observed abundance of *R. italica*, which represents an index of frog abundance (Barke et al. 2017). Microclimatic features (temperature, humidity and illuminance), biotic features (presence/absence of the seven prey species), month

Table 1. Data of explored subterranean environments. For each site are shown: longitude, latitude and elevation, orientation of the main entrance, typology (natural (N), semi-natural (SN), artificial (A)), total development of the inner environment (Max area) and length explored in this study (linear meters), maximum number of observed frogs during a single survey, deepest observed frog (linear distance from cave entrance). *The length of last cave sector was only 1 m as a syphon blocked the passage. **This is a provisional data as the cave is still interested by speleological exploration. "X" means that for the artificial site no information is available for its total length.

Site	Longitude	Latitude	Elevation	Orientation	Typology	Max area (m)	Explored area (m)	Maximum number of observed frogs	Deepest observed frog (m)
Site1	11.21	43.87	227	S-E	N	12	12	2	9
Site2	11.13	43.91	286	N	N	15	10*	2	9.5
Site3	10.82	44.00	948	N	SN	12	12	8	12
Site4	10.82	44.00	853	N-E	SN	15	15	1	11.5
Site5	10.82	44.00	850	N-E	SN	21	21	1	10.45
Site6	10.85	44.04	744	N-E	A	X	18	2	12.8
Site7	11.16	43.91	699	N-E	N	6	6	2	5.9
Site8	11.15	43.91	715	N	N	52	42	2	20.5
Site9	11.15	43.97	492	N-W	N	78**	60	12	24.5

of survey and sector depth were used as independent variables; sector and cave identity were used as random factors.

We used Linear Mixed Models (LMM) to identify whether differences in spatial use of caves occur between age classes or sexes. To assess differences between adults and juveniles, we run two LMMs using age class and month of survey as independent variables, while both cave and sector identity were included as random factors. The distance of individuals from cave entrance, and the vertical position of frogs (i.e., height above the cave floor) were the dependent variables of the two LMMs. This procedure was repeated on adults only, replacing the independent variable age class with sex, in order to detect differences between adult males and females.

All analyses were performed in the R environment using packages lme4, lmerTest, nlme and unmarked (Fiske and Chandler 2011, Douglas et al. 2015, Kuznetsova et al. 2016, Pinheiro et al. 2016, R Core Team 2016).

Results

We performed a total of 765 surveys within 66 cave sectors (missing data due to unfavorable climatic condition represent 4.42%), recording 122 detections of *Rana italica* (60 females, 9 males, 38 juveniles and 15 unsexed individuals) (Table 1); on average (\pm Error Standard; ES), we observed 1.14 ± 0.21 individuals per survey (Fig. 2).

The abundance of *R. italica* was strongly related to the depth of the sector ($F_{1,98.28} = 30.40$, $P < 0.001$), air temperature ($F_{1,716.39} = 4.29$, $P = 0.038$), illuminance ($F_{1,143.82} = 8.01$, $P = 0.005$) and to the presence of two insect species, *Dolichopoda laetitia* ($F_{1,645.63} = 7.71$,

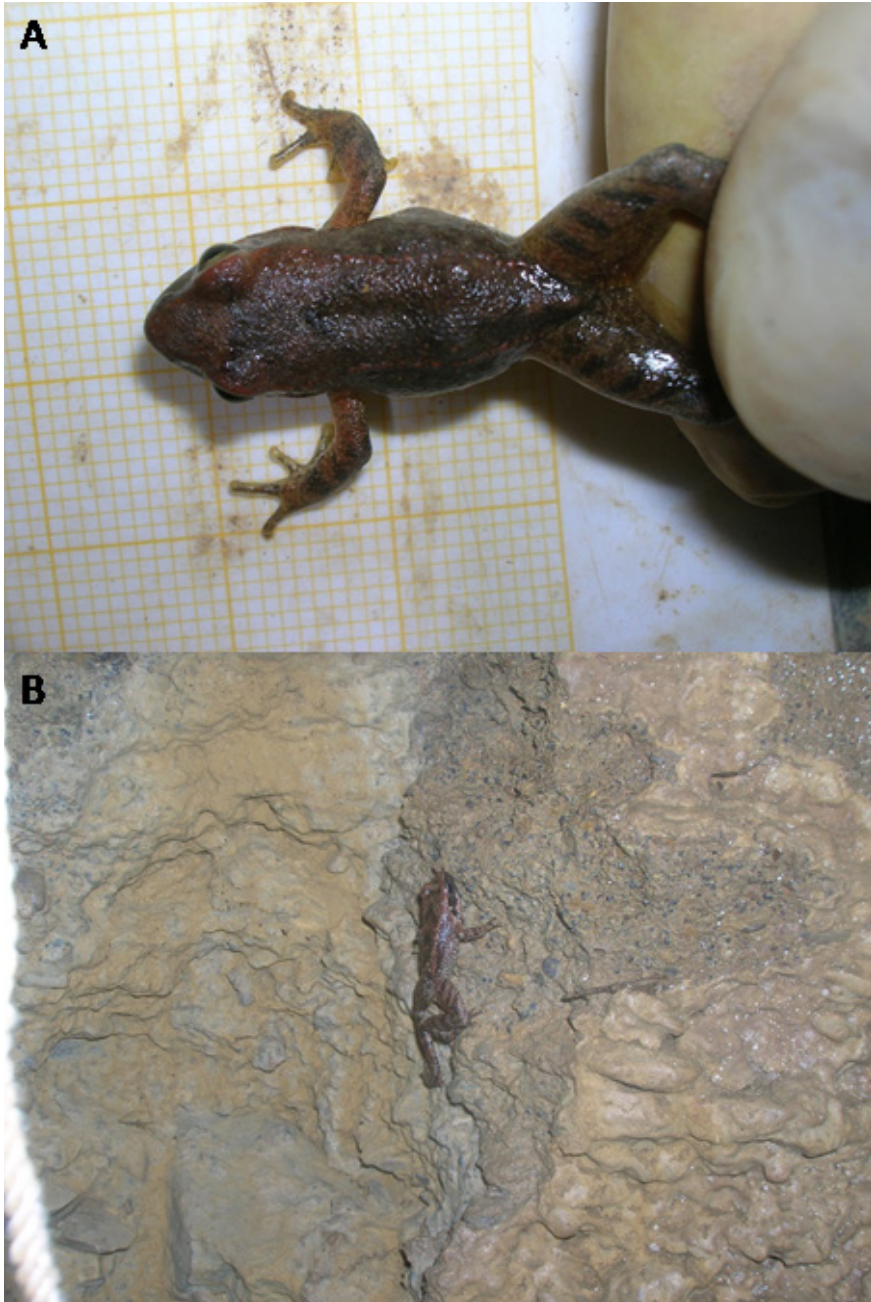


Figure 1. Two juveniles of *Rana italica*: a) during the measurement of SVL and b) climbing cave walls.

$P = 0.006$) and *Limonia nubeculosa* ($F_{1,730.17} = 5.50$, $P = 0.019$). Overall, frogs were more abundant in shallow sectors with less light and warmer temperature, and with the presence of both *D. laetitia* and *L. nubeculosa*. Furthermore, we detected significant differences of

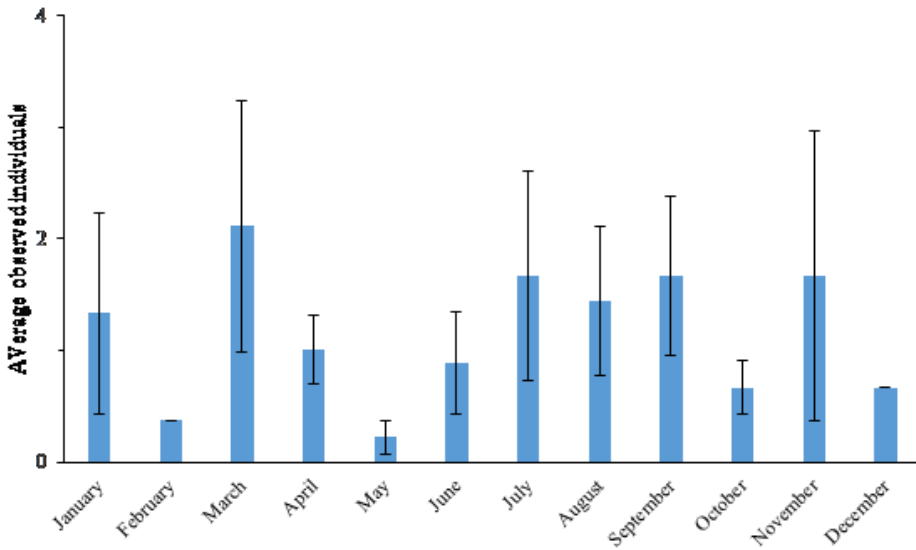


Figure 2. Average monthly observation (\pm ES) of *Rana italica* within studied caves.

frog abundance among months ($F_{11,704,58} = 2.46$, $P = 0.005$). March and November were the survey months in which we recorded the highest frog abundance (Fig. 2). All other considered variables were not significant ($P > 0.17$).

The distance from the cave entrance did not show significant differences between age classes, without differences among survey months (age class: $F_{1,67} = 0.80$, $P = 0.374$; month: $F_{9,67} = 1.04$, $P = 0.417$) (Fig. 3A), nor between sexes (sex: $F_{1,30} = 0.56$, $P = 0.46$; month: $F_{9,30} = 0.45$, $P = 0.894$) (Fig. 3B). The use of walls was significantly different between age classes ($F_{1,67} = 4.92$, $P = 0.03$) (Fig. 3C) without differences among survey months ($F_{9,67} = 1.86$, $P = 0.073$); adults generally occupied higher position compared to juveniles. Within adults, we did not detect differences between males and females (month: $F_{9,30} = 1.07$, $P = 0.413$; sex: $F_{1,30} = 0.01$, $P = 0.912$) (Fig. 3D).

Discussion

Rana italica was observed in subterranean environments throughout the year, even though the number of observations varied between months (Fig. 2). The abundance of *Rana italica* in subterranean environments was higher in areas close to the cave entrance, but deep enough to show almost no light. These particular areas correspond to the twilight zone (Culver and Pipan 2014), where external influence is moderate and abundance of non-strictly cave-dwelling species is the highest (Lunghi et al. 2015, Lunghi et al. 2017). The amount of incoming light represents an important factor for *R. italica*, as it influences both presence and abundance of the species in subterranean environments (Lunghi et al. 2017). Stream frogs are mostly nocturnal, and preference

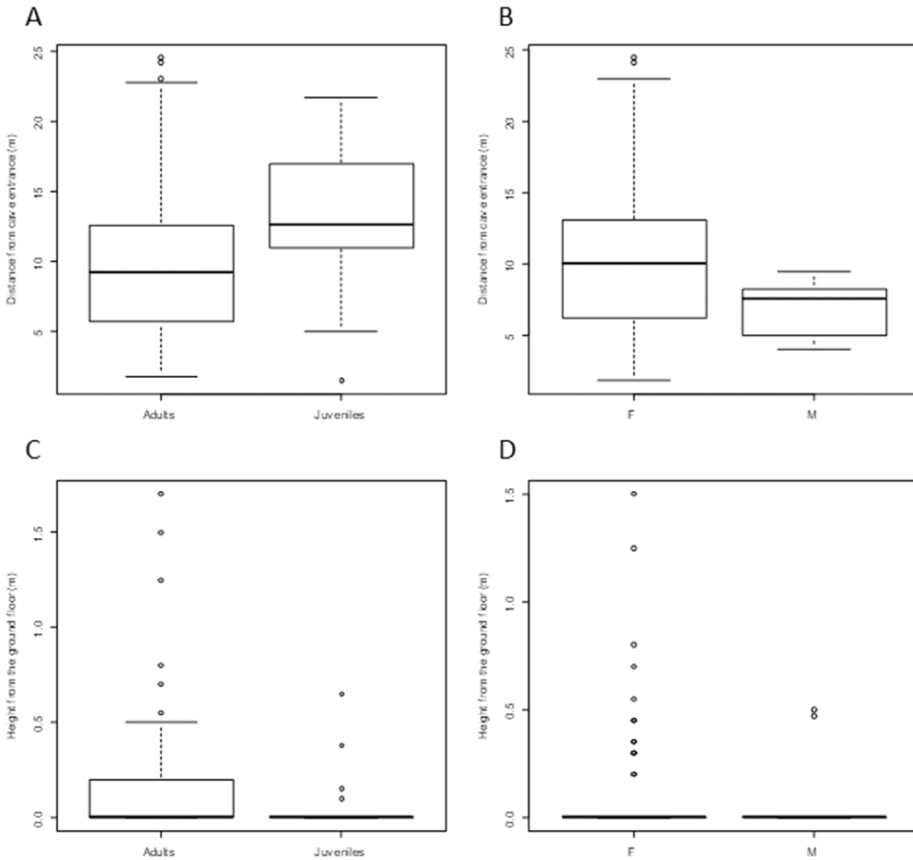


Figure 3. Boxplots indicating differences in the use of cave spaces. Difference between **A** age classes (Adults/ Juveniles) and **B** adult sexes (Females/Males) in the use of the subterranean surface area; differences between **C** age classes and **D** adult sexes in the use of cave walls. Diagonal bar inside the box represents the median.

for twilight areas might help them to avoid predators (Vanni and Nistri 2006); however, we cannot exclude that the selection of sectors without light may be also related to the avoidance of harsher climatic conditions (hot and dry), which are present close to the surface (Hetema et al. 2012, Everall et al. 2014). Temperature was an additional parameter strongly related to abundance of *R. italica*. Frogs were more abundant in relatively warm cave sectors. The temperature of cave sectors is the result of complex interactions between external temperature, mean annual temperature, and air circulation of karstic systems (Badino 2004, Badino 2010, Lunghi et al. 2015). In winter, temperature is highest far from the surface, thus these sectors represent ideal shelters during hibernation. Conversely, in summer the temperature is highest close to cave entrance where prey richness is highest (Manenti et al. 2015, Lunghi et al. 2017). Indeed, the abundance of frogs was positively related to the presence of invertebrates such as the cave cricket *Dolichopoda laetitiae* and the fly *Limonia nubeculosa*. Stream frogs are

generalist predators of small invertebrates, and these two insects are often abundant in subterranean environments, therefore they have a key role in sustaining subterranean trophic networks (de Pasquale et al. 1995, Lavoie et al. 2007, Manenti et al. 2015). Recent studies underlined that the occurrence of the cricket *D. laetitiae* is strongly related to inner cave temperature, as this cricket prefers cave areas with relatively warm temperature (Lunghi et al. 2015, Lunghi et al. 2017). No particular information is available on the seasonal occupancy of caves by *Limonia nubeculosa*. Therefore, the relationship between *R. italica* abundance and the occurrence of these species is likely to represent the result of trophic interactions.

As already observed for other amphibians present in subterranean environments, individuals of *R. italica* probably face a trade-off between the selection of suitable conditions, and the need of trophic resources. Thus, frogs occupy cave portions that are deep enough to have a suitable microclimate and lack of predators, but that are not too far from feeding areas (Ficetola et al. 2013, Lunghi et al. 2016). The abundance and the frequency of individuals found in subterranean environments suggest that further investigations should be performed on the trophic role and the impact that *R. italica* may have on the cave food web. In a study performed on the pickerel frog (*Rana palustris* (LeConte, 1825)) involving analyses with both stable isotopes and stomach contents, authors identified a significant predator activity of this species within the cave habitat (Fenolio et al. 2005). In our study, the correlation between *R. italica* and the two insect species suggests that some trophic relationships may occur, as it is possible that frogs are more abundant in cave sectors with the presence of potential prey. On the other hand, it is also possible that some unrecorded features (e.g., availability of resources) positively influence the abundance of multiple species, including both frogs and insects. Indeed, as recently underlined for aquatic subterranean sites, amphibian predators exploiting subterranean environments not only contribute to the top-down control of prey, but also provide an important subsidy in terms of energy for such environments (Barzaghi et al. 2017).

Frog distribution within caves was similar between age classes and sexes (Fig. 3A–B), suggesting that there is neither spatial segregation between age classes, nor between adult sexes. Actually, no information exists on a possible spatial segregation between individuals of *Rana italica*. In our study, the abundance and diversity of potential prey observed in subterranean environments likely promote the co-existence of different individuals. However, the use of the subterranean space by *R. italica* was not limited to the cave floor, as frogs often climbed walls (Figs 1B, 3C–D). This particular behavior, which is generally unusual for frogs, might be compared to observations reported for other two anuran species. In green frogs (*Pelophylax synklepton hispanicus* (Bonaparte, 1839)) some adults were observed preying on high tree branches (Mori et al. 2013). In artificial subterranean environments, the Iberian frog (*Rana iberica* Boulenger, 1879) has been observed climbing up the walls, probably looking for shelters (Rosa and Penado 2013). In our study, adults were observed more frequently being up higher along cave walls compared to juveniles (Fig. 3C); this divergence could be related to a different climbing ability and/or behavior between age classes.

Conclusions

This study represents the first analysis on the factors related to the abundance and the spatial use of *Rana italica* in subterranean environments. The obtained results enable us to hypothesize that subterranean abundance of *R. italica* is mostly related to foraging activities, thus likely make this species one of the top predators of the twilight zone. Our study underlines that the trophic role of terrestrial predators exploiting subterranean environments should be taken into account for the ecological comprehension of determinants which have promoted the colonization of caves. Nonetheless, subterranean environments may also be of key importance to maintain the viability of anuran populations in the light of the occurring global warming.

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