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Do Salamanders Limit the Abundance of Groundwater Invertebrates in Subterranean Habitats?

Raoul Manenti [1,2,*], Enrico Lunghi [3,4], Benedetta Barzaghi [1], Andrea Melotto [5], Mattia Falaschi [1] and Gentile Francesco Ficetola [1,6]

1   Department of Environmental Science and Policy, Università degli Studi di Milano, via Celoria, 26, 20133 Milano, Italy; benedetta.barzaghi@unimi.it (B.B.); mattia.falaschi@unimi.it (M.F.); francesco.ficetola@unimi.it (G.F.F.)
2   Laboratorio di Biologia Sotterranea "Enrico Pezzoli", Parco Regionale del Monte Barro, 23851 Galbiate, Italy
3   Key Laboratory of the Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beichen West Road 1, Beijing 100101, China; enrico.arti@gmail.com
4   Museo di Storia Naturale dell'Università degli Studi di Firenze, sede "La Specola", Via Romana 17, 50125 Firenze, Italy
5   Centre for Invasion Biology, Stellenbosch University, Matieland 7602, South Africa; mel8@hotmail.it
6   LECA, Laboratoire d'Ecologie Alpine, CNRS, The Grenoble Alpes University and The Savoie Mont Blanc University, F-38000 Grenoble, France
*   Correspondence: raoulmanenti@gmail.com

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Abstract: Several species of surface salamanders exploit underground environments; in Europe, one of the most common is the fire salamander (*Salamandra salamandra*). In this study, we investigated if fire salamander larvae occurring in groundwater habitats can affect the abundance of some cave-adapted species. We analyzed the data of abundance of three target taxa (genera *Niphargus* (Amphipoda; Niphargidae), *Monolistra* (Isopoda; Sphaeromatidae) and *Dendrocoelum* (Tricladida; Dedrocoelidae)) collected in 386 surveys performed on 117 sites (pools and distinct subterranean stream sectors), within 17 natural and 24 artificial subterranean habitats, between 2012 and 2019. Generalized linear mixed models were used to assess the relationship between target taxa abundance, fire salamander larvae occurrence, and environmental features. The presence of salamander larvae negatively affected the abundance of all the target taxa. *Monolistra* abundance was positively related with the distance from the cave entrance of the sites and by their surface. Our study revealed that surface salamanders may have a negative effect on the abundance of cave-adapted animals, and highlited the importance of further investigations on the diet and on the top-down effects of salamanders on the subterranean communities.

Keywords: cave biology; prey; hypogean; underground; stygofauna; *Monolistra*; Sphaeromatidae; *Niphargus*; flatworm; aqueduct; seepage

1. Introduction

Salamanders represent an important fraction of aquatic and terrestrial biomass in several environments. Salamanders typically display a life cycle involving aquatic larvae and terrestrial adults. However, several peculiar adaptations to a total terrestrial or to a complete aquatic life evolved separately in different salamanders' lineages allowing the exploitation of a large variety of environments [1]. In both cases, salamanders often retain the role of keystone predators, affecting the structure of the communities in different aquatic and terrestrial habitats [2]. In temporary ponds, salamanders are known to regulate the nutrient flows within aquatic food webs by affecting the abundance of zooplankton and tadpoles [3]. In forests, salamanders are often abundant mesopredators

that can strongly affect the abundance and composition of invertebrate communities, sometimes even mediating the rates of leaf litter decomposition [4]. Salamanders' predatory activity can also determine trophic cascades (such as changes in the trophic web across two or more links) especially in communities based on detritus [5]. For example, the red-backed salamander (*Plethodon cinereus*) is an abundant predator on springtails, mites, and other small prey invertebrates [6], which in turn feed upon a large fungal biomass [7]. Thus, red-backed salamanders play top-down effects on fungal communities of forest floors [8].

Among the environments with detritivore-based trophic webs, groundwaters provide a promising research field that deserves to be implemented for different reasons. First of all, groundwater represents the major source of potable water supply for humans and, globally, is the largest source of available freshwater [9,10]. Second, underground freshwater environments such as aquifers, hyporheic zones, and cave rivers can be of particular interest to understand processes shaping global biodiversity. In these environments, ecological variation is weak compared to surface habitats, and this facilitates studies assessing mechanisms that allow colonization by animals and the differentiation of colonizers that often follows [11,12]. Although the majority of studies on groundwater fauna report findings and descriptions of new species, there is an increasing interest in understanding the evolutionary processes involved in cave colonization and the distribution of cave adapted animals [13,14]. Most studies on animals inhabiting groundwater refer to "stygobionts", i.e., those animals that evolved specific adaptations to underground freshwater habitats, in which they spend their entire life-cycle [15]. Among them there are at least 13 species or subspecies of salamanders that are considered obligate cave-dwellers and display typical morphological adaptations (e.g., eyeless and depigmentation) to the subterranean environment [16]. These salamanders are often fully aquatic and occupy the top predator role in groundwaters. However, non-obligate cave-dwelling salamander species can also play a fundamental role in shaping cave food webs. Several species of surface salamanders are known to exploit underground environments where they can feed on invertebrates, guano, or other urodeles [17–20]. Moreover, some of them often breed in subterranean rivers and streams where their larvae are able to complete the entire lifecycle [21]. An increasing number of studies are showing their ecological and evolutionary importance. As an example, they can help us understand the dynamics of novel habitat colonizations and provide useful insights to understand the relative role of phenotypic plasticity and local adaptations [22]. Moreover, as they can reach high abundances and show well defined patterns (i.e., seasonal, ecological) of cave exploitations, they can exert important roles on the community inhabiting the surrounding of the cave entrance and the twilight zone [23,24].

In the last years, a growing number of studies has investigated the ecology of fire salamanders (*Salamandra salamandra*) breeding in underground environments. This species is an ovoviviparous widespread amphibian in Europe that shows high ecological plasticity in the choice of breeding sites [25–27]. This salamander can breed in numerous subterranean environments; larvae can be found in natural caves streams and pools, artificial hypogean springs and flooded mines where they may reach high densities [28]. Generally, in groundwaters, fire salamander larvae are found within the first 5–30 m from the cave entrance; however, records of larvae in deeper areas (>100 m) are also reported [21,29]. In most of the groundwater sites where fire salamander breeds, larvae occupy the top predator position [30,31], however, prey is often rare and food scarcity poses major constraints to their development [32].

An aspect that is still not well understood is the role played by fire salamander larvae on stygobiont fauna. The exploitation of groundwater by animals normally occurring at the surface can determine changes in the composition of stygobiont communities [33]. For example, a recent study considering natural and artificial spring habitats revealed that the occurrence of fire salamander larvae limits the occurrence of the stygobiont *Niphargus thuringius* at the interface between groundwater and surface streams [34].

With this study we aim to assess if the occurrence of fire salamander larvae in subterranean habitats affects the abundance of cave adapted animals. We predict that, irrespective to the distance from the

entrance, groundwater sites with fire salamander larvae show a lower abundance of stygobiont species than groundwater sites without larvae.

2. Materials and Methods

2.1. Sampling Design

Between 2012 and 2019, we performed repeated sampling of subterranean aquatic fauna across multiple caves and artificial subterranean habitats with streams or pools. Each considered site was visited at least twice during the same season of the same year; we only considered in analyses sites where the occupancy of fire salamander larvae did not change between surveys (i.e., salamander larvae were always present or absent during the successive surveys). The subterranean sites (Figure 1) are located between the districts of Como, Lecco, Bergamo, and Monza and Brianza of Lombardy and of La Spezia in Liguria (NW-Italy).

To obtain preliminary information on caves (i.e., location, development) we used the data from the cave cadasters of Lombardy and Liguria. The artificial subterranean sites considered here were artificial subterranean springs, draining galleries of catchment (the so called 'bottini'), and artificial mines. To localize the artificial subterranean sites, we used information available in studies on subterranean fauna [35] and local information on mine activity.

Visual encounter surveys were performed to assess salamander larvae occurrence and stygobiont abundance. Water depth and distance from the entrance were also measured. In all sites we sampled the largest pool or waterbody that we found and the other pools or streams that occurred. In streams, we randomly choose one or more sections of the watercourse from the cave entrance to the deepest part that we reached. Overall, we performed 386 visual samplings, surveying 117 sites (pools and distinct subterranean stream sectors) within 17 natural caves and 24 artificial cavities.

We searched both stygobiont fauna and fire salamander larvae by employing standardized visual encounter surveys, during which each pool or stream's sector was actively investigated with a constant effort of 3 min/m^2 [36]. The detection probability of fire salamander larvae is generally high, especially during nights in surface environments and in groundwaters and visual observations that allow to detect the species occurrence with confidence >0.95 [37], thus false absences in our analyses are unlikely.

We assessed the potential role of fire salamander larvae on the abundance of three taxa of stygofauna: amphipods of the genus *Niphargus*, isopods of the genus *Monolistra*, and planarians of the genus *Dendrocoelum* (Figure 2). All these animals show features typical of cave-adapted species, such as eyeless and depigmentation. *Monolistra* isopods generally feed on detritus and biofilms occurring on the substrate and composed of fungi and bacteria [38,39]. *Niphargus* crustaceans show a generalist diet comprising both plant debris and other arthropods, and display both a detritivore and a predatory/cannibalistic behavior [40,41]. Planarians are predators and can hold the highest position of the food web in small interstitial groundwater habitats or where salamanders do not occur. In this study, we focused at the genus level and, for each genus, we included multiple species in the analyses. Multiple *Niphargus* species are present in all the groundwaters of the study area but high confusion regarding their taxonomy exists [39]. In the study area, *Monolistra* crustaceans include different species that occupy different distinct karst areas; we investigated caves in the range of *M. pavani*, *M. bergomas*, and *M. julia*. Only a limited number of planarians of the genus *Dendrocoelum* are currently described for Italian caves [42], and during our investigations we have recorded a higher number of localities at which these flatworms occur.
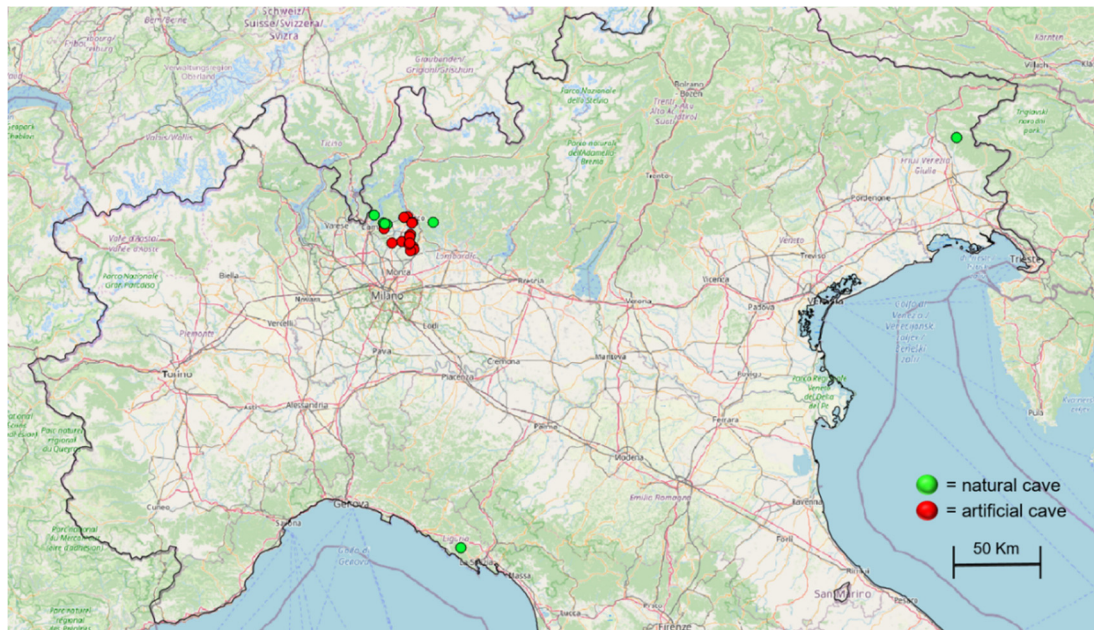
Figure 1. Sampling caves considered in this study. Caves are divided into natural (green dots) and artificial (red dots). Due to geographic proximity most of the sites are superimposed.

2.2. Statistical Analyses

We used generalized linear mixed models (GLMMs) to assess the relationships between the abundance of the target taxa, salamander occurrence, and habitat features. Generalized mixed models yield reliable estimates of the relationships between the relative abundance of animals and environmental conditions [43]. Before performing GLMMs we checked correlations between all the variables. We performed three distinct GLMMs, one for each stygobiont taxon. Sites outside the range of the *Monolistra* species were excluded from the analysis focusing on this genus (Supplementary Table S1), to avoid bias related to biogeographical patterns. As dependent variables, we considered the number of active individuals of the target taxa observed for each site at each sampling occasion. As an independent variable we used the occurrence of fire salamander larvae, the distance from the cave entrance, and the maximum water depth; we included also the area of the sites as covariate. As random factors we considered the cavity in which we sampled the subterranean pools or the streams and the year of sampling. We built models using negative binomial distribution (type I). For each GLMM we tested all combinations of explanatory variables for multicollinearity using the variance inflation factor (VIF); all VIF were below 2. We assessed significance of variables in GLMMs using a likelihood ratio test.

GLMMs were run in R environment (R Development Core Team 2018) using the packages lmerTest [44], glmmTMB [45], and car [46].

3. Results

Fire salamander larvae occurred in 41 sites (23 caves). The most widespread cave-dwelling taxon was the genus *Niphargus* that was detected in 48 sites (23 caves). By contrast, the planarians of the genus *Dendrocoelum* were more localized, occurring in 28 sites (11 caves), while crustaceans of the genus *Monolistra* were recorded in 25 sites (four caves only) and 47 sampling occasions (Table S1). Although localized, *Monolistra* reached the maximum abundance recorded at a single site with 106 individuals. Instead, maximum abundance at a single site was 56 individuals for *Dendrocoelum* and 20 individuals for *Niphargus*. The proportion of surveyed microhabitats occupied per cave varied consistently; generally, in the caves where we detected *Monolistra* occurrence, these isopods were detected in 90% of the sites, while *Niphargus* and *Dendrocoelum* were detected in a substantially minor fraction of microhabitats.

We detected *Monolistra* only in natural caves and in natural microhabitats, while both *Niphargus* and *Dendrocoelum* occurred also in different artificial pools of draining galleries.

GLMMs revealed that the occurrence of fire salamander larvae played a significant effect on the abundance of all the target taxa (Table 1). All target taxa showed a reduced abundance in sites with salamander larvae (Figure 3). The abundance of *Niphargus* was positively related to the maximum depth of the sites with higher densities occurring in deeper sites (Table 1). For *Monolistra* the analysis also revealed a tendency to occupy habitats farther from the entrance and with larger area (Table 1).
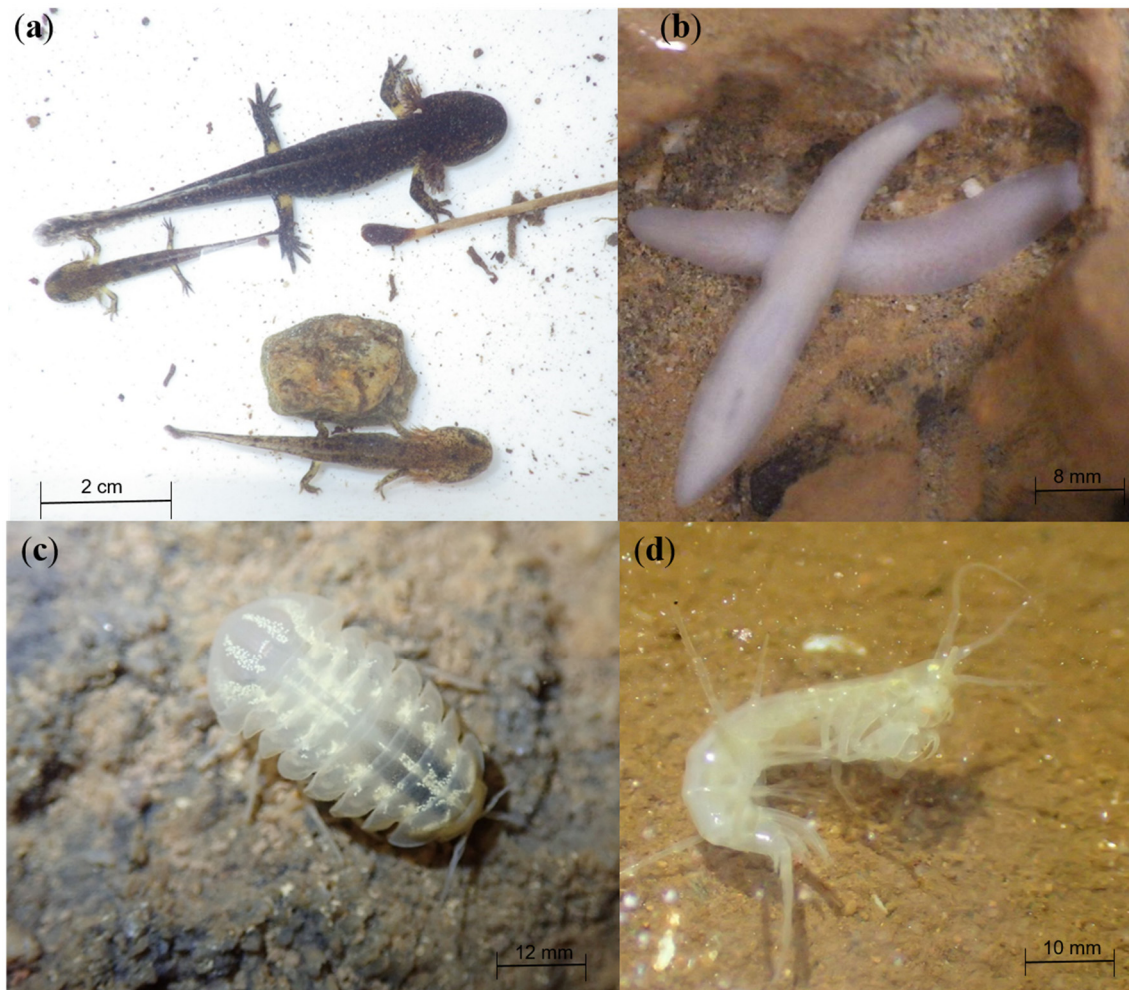
Figure 2. Examples of the taxa considered in the study: (**a**) *Salamandra salamandra* larvae at different stages; (**b**) a *Dendrocoelum* flatworm from the Pignone cave (Liguria); (**c**) an isopod crustacean of the genus *Monolistra* (*Monolistra pavani*); (**d**) an amphipod crustacean of the genus *Niphargus* (*Niphargus thuringius*). Credits R. Manenti.
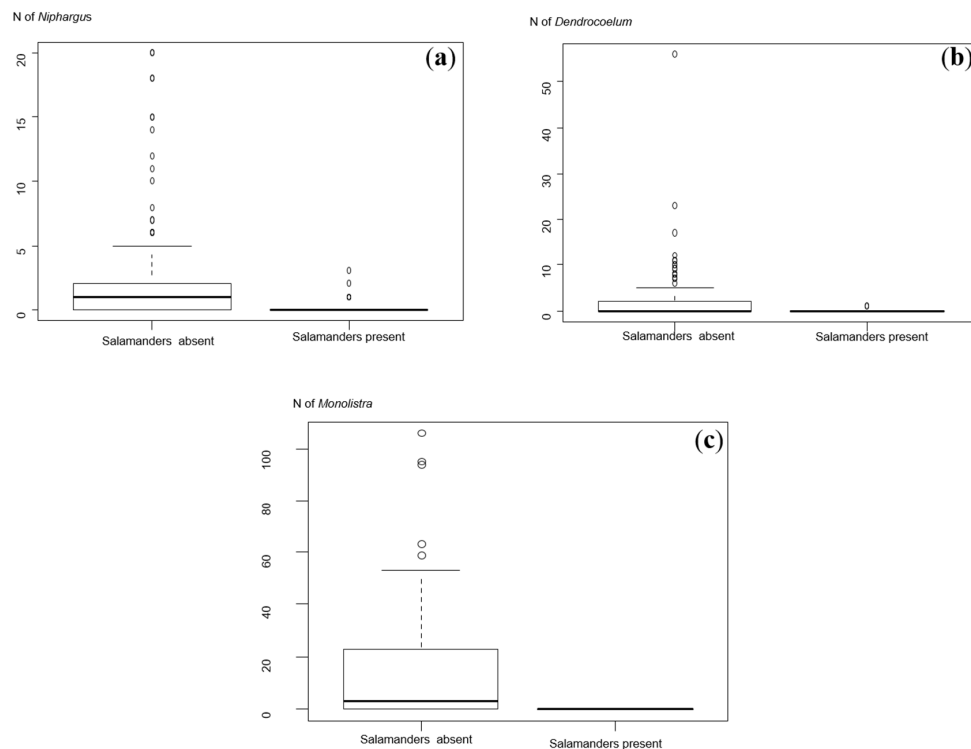
Figure 3. Boxplots of relationships between the occurrence of fire salamander larvae and abundance of the stygobiont target taxa: (**a**) amphipods of the genus *Niphargus*; (**b**) planarians of the genus *Dendrocoelum*; (**c**) isopods of the genus *Monolistra*.

Table 1. Results of the likelihood ratio test on generalized linear mixed models (GLMMs) assessing the relationship between the presence of fire salamander larvae and environmental variables with the abundance of the three target stygobiont taxa. Significant relationships are in bold.

	Variables	Estimate	SE	χ2	P
Niphargus					
	Fire salamander larvae	**−3.34**	**0.44**	**78.31**	**<0.001**
	Distance from surface	<0.01	<0.01	0.01	0.94
	Maximum water depth	**0.01**	**<0.01**	**4.29**	**0.03**
	Surveyed area	−0.05	0.05	1.043	0.30
Dendrocoelum					
	Fire salamander larvae	**−2.39**	**0.84**	**11.53**	**<0.01**
	Distance from surface	<0.01	<0.01	1.35	0.24
	Maximum water depth	<−0.01	0.01	0.57	0.44
	Surveyed area	0.06	0.07	0.74	0.38
Monolistra					
	Fire salamander larvae	**<−0.01**	**<0.01**	**6.24**	**0.01**
	Distance from surface	**<0.01**	**<0.01**	**4.71**	**0.02**
	Maximum water depth	<0.01	<0.01	0.67	0.41
	Surveyed area	**<0.01**	**<0.01**	**6.98**	**<0.01**

4. Discussion

This is the first study that investigated the relationship between facultative cave-breeding salamanders and the relative abundance of invertebrate fauna adapted to groundwaters. Our results indicate that the occurrence of fire salamander larvae in groundwaters may limit the density of different stygobiont animals such as crustaceans and planarians, showing that these animals can shape the diversity of fauna in groundwaters, at least nearby the surface. Previous studies have shown that salamander occurrence in caves is favored by some cave features, such as the stability of habitat (water permanence), the absence of predators, and the availability of resources [47,48]. Caves and other

subterranean environments with groundwater may offer more stable breeding habitats, with a more regular hydroperiod, than surface streams and creeks, which especially in karst landscapes may be subjected to strong variation depending on the amount of rainfalls [26]. Moreover, cave pools are usually predator-deprived environments and can be considered as safe habitats for the fire salamander larvae [31]. However, these environments also harbor low densities of invertebrate prey, especially when compared to surface breeding sites, posing a constraint to larval development [49].

Fire salamander larvae are generalist predators that can prey upon a large range of invertebrates [50–52]. In groundwater habitats that are close to surface, animals from outside, like dipterans and crustaceans, may occur and become prey of fire salamander larvae [33,53]. However, stygobiont species can also constitute a useful resource and be opportunistically preyed. For these animals, the trophic perspective is reversed because, compared to deepest sectors, the underground habitats close to the surface can be richer in terms of available food [54–56]. Thus sectors close to the surface and surface habitats themselves can provide useful trophic resources for stygobionts which, in favorable seasons or with particular environmental conditions, can occupy springs or move closer to the cave entrance. At the same time, areas at the boundary between underground and surface environments can be more risky in terms of climatic variation (they are unstable compared to deep subterranean habitats) and predator occurrence [57,58]. Our results suggest that when a top-predator occurs in subterranean habitats, it may severely limit the abundance of stygobiont fauna, since all the three target taxa considered in this study showed a significant lower abundance in sites with fire salamander larvae. To assess the effect of direct predation by fire salamander larvae on stygofauna further investigations using stomach flushing or stable isotope analysis are necessary.

In particular, we observed a negative relationship between salamander larvae and the abundance of *Niphargus* crustaceans and *Dendrocoelum* planarians. With more than 430 described species at the global scale, *Niphargus* is the most diverse genus of freshwater amphipods [59–61]. It is widespread and primarily inhabits groundwaters, but several *Niphargus* species/populations live in subterranean habitats at the interface with the surface and can more or less occasionally exploit epigean environments like springs and streams [34,61,62]. Even if eyeless, *Niphargus* species retain the ability to detect light [63] suggesting that the connection with surface environments and exploitation of transitional habitats can be important for these crustaceans. Salamander larvae can exhert major predatory pressure on these animals; it is also important to consider that the occurrence of salamander larvae is seasonal and, even if their development can be quite long [64,65], periods in which larvae are absent are likely to occur. If we consider also that the biomass of laid larvae is generally higher than that of metamorphosing one [32], it is possible that the subsidization by fire salamander can also have effects on *Niphargus* and other organisms when the predation pressure is not present or present only in adjacent microhabitats. Further investigations could be performed by surveying the same subterranean habitats when there will be no fire salamander larvae inside. *Niphargus* abundance was also positively related to water depth. Other than hosting a higher water volume to be surveyed, deeper pools can provide more shelters during water flow and host more organic debris.

Dendrocoelum planarians are predators of annelid, crustaceans, and other invertebrates [66,67]; very few studies are available for subterranean species and there is lack of ecological information on factors favoring their abundance and distribution. Only a few cave species with very narrow ranges are currently known in Italy [42]. *Dendrocoelum* planarians, when fire salamander larvae occur, can be considered as mesopredator; our data suggests however that the effect of fire salamander larvae is similar in planarians, *Niphargus*, and detritivore *Monolistra* as well. Predation of salamanders on subterranean planarians has been observed in the case of the Barton Springs salamander (*Eurycea sosorum*), suggesting that planarians may be a significant but ignored prey item for aquatic salamander species/larvae [68].

The abundance of the crustaceans of the genus *Monolistra* was not only negatively related to fire salamander larvae occurrence, but also to the distance of the sites from the cave entrance. In particular we observed more *Monolistra* individuals in sites more distant from the surface. *Monolistra* is a genus of Sphaeromatidae that probably colonized caves form marine habitats [69]; it is possible that its

occurrence is linked to older and stable aquifers and less linked to small groundwater sites close to the surface. Moreover, we also detected also a positive significant relationship between *Monolistra* abundance and the area of the pools. This variable might reflect the sampling effort as well as the surveyed suitable habitat. In some sites we observed high abundances of *Monolistra* that can constitute an important portion of the invertebrate biomass of groundwater. Further researches on these stygobionts are needed to understand their patterns of subterranean habitats exploitation.

The higher predation occurring close to surface may be one of the factors that limit the exploitation of interface habitats by groundwaters dwelling species when underground conditions are similar to those occurring in surface (e.g., during night or in intermediate seasons). However, the negative relationship observed between salamander larvae and stygofauna abundance could also be caused by non-consumptive effects of fire salamander and the landscape of fear generated by its occurrence. Moreover, analyses on interspecific/intraspecific interactions between stygobionts are required to understand how multispecies dynamics affect the abundance of the different invertebrate species. Finally, comparisons between the abundances in open pools/stream sectors and substrate/rocks interstices could provide further insights on the role played by microhabitat heterogeneity.

5. Conclusions

Salamander larvae can be a major predator for cave-adapted animals, with a keystone role at least in subterranean areas closer to the surface. However, these environments can be heavily impacted by ongoing climate changes, such as temperature increase and reduction of water availability, that may promote an increase in the use of caves by surface animals [70]. An increasing exploitation of caves by salamanders can have consequences on cave-adapted animals; thus understanding the role played by salamanders as predators can be central for the management of subterranean biodiversity at a broad scale. When in caves salamanders occupy the top predator level, thus they may have a top-down effect on other organisms not considered in this study. Future comparisons between the biofilms occurring in subterranean sites with and without fire salamander larvae could provide further insights on their cascading effects on cave trophic web.

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