**Biphasic predators provide biomass subsidies in small freshwater habitats: a case study of spring and cave pools.**

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**Keywords**

**Summary**

1) There is increasing attention on how biomass exchanges determine linkages between ecosystems. However, there is limited information on the factors determining the direction and overall magnitude of energy flow, especially in systems where primary producers are limited.

2) In this study we compared two aquatic ecosystems (cave and spring pools) using the biomass exchanges driven by a biphasic predator, the fire salamander (*Salamandra salamandra*).

3) Between 2013 and 2014, we monitored 21 fire salamander breeding sites (12 cave pools and 9 spring pools) and we quantified the larval biomass input-export.
The balance between input and export was related to several abiotic and biotic variables.

4) *Salamandra salamandra* larvae constituted a major component of the animal biomass in both cave and spring pools, and were the most abundant top predator. Light was the most important parameter in constraining predator biomass; biomass export was lowest in the sites that received less light. In cave pools, the biomass balance was negative, while it was positive in spring pools.

5) Our study demonstrates that cave pools are a system with much lower productivity than springs, and their functioning strongly depends on the input of external resources. Predator occurrence may constitute a major trophic subsidy in poorly productive environments. Gradients of abiotic variables such as light incidence can constrain population size of predators, determining strong variation of their biomass balance.

**Introduction**

Biomass production and exchange are key properties determining ecosystem functioning (Wallace *et al.*, 2015). General theoretical models affirm that all the trophic levels of an ecosystem have a positive response to productivity gradients (Chase, 2003). Resource supply is a fundamental constraint on the biomass and production at all trophic levels. Unravelling the processes that determine the amount of available resources in ecosystems is pivotal in understanding their functioning, and also allows assessment of the role of environmental conditions that affect the outcome of complex biotic interactions (Kiffney, Richardson & Bull, 2003;  Knapp, 2005;  Kupisch *et al.*, 2012). One aspect that is gaining increasing attention in functional ecology is how the exchanges from surrounding ecosystems may affect consumer and predator biomass and abundance in a certain environments (Iskali & Zhang, 2015;  Venarsky *et al.*, 2014). Exchanges of detritus, trophic resources, prey and predators between nearby ecosystems play important roles in the functioning of ecosystems (Kreps, Larson & Lodge, 2016;  Regester, Lips & Whiles, 2006).

Spatial and functional linkages between two ecosystems may occur because of abiotic processes that spread or displace organic matter, and through the active or passive moving of organisms between different habitats (Kraus, Pletcher & Vonesh, 2011). Many taxa exploit multiple ecosystems during their life cycle. For instance, several lucifugous organisms animals (such as that avoid light) exploit both underground (such as habitats occurring underground) and outdoor (such as habitats occurring above ground) habitats during their life cycles. The movements of these animals between underground and outdoor environments (Culver & Pipan, 2009;  Lunghi, Manenti & Ficetola, 2015;  Manenti, Lunghi & Ficetola, 2015) likely have strong effects on the fluxes of resources between these adjacent ecosystems (Fenolio *et al.*, 2006;  Fenolio, Graening & Stout, 2005;  Iskali & Zhang, 2015;  Lunghi, Manenti & Ficetola, 2014). Outside caves, species with biphasic life cycle have a comparable role. For example, many insects and amphibians lay eggs and larvae in water. After metamorphosis they show fully terrestrial adult stages (Earl & Semlitsch, 2012;  Eitam, Blaustein & Mangel, 2002), and the flow of individuals can determine a relevant flow of biomass between terrestrial and aquatic ecosystems. Such a flow can be positive (i.e. with biomass input into aquatic ecosystems higher than biomass export to terrestrial environments), or negative, with input lower than export. Despite the importance of biomass flow for small and nutrient-poor ecosystems, there is limited information on the factors determining the direction and overall magnitude of such flows (Reinhardt *et al.*, 2013;  Schneider, Christman & Fagan, 2011).

Ecosystems in which light availability is a constraining factor may provide new insights into some of these interactions. Recent studies show that algal production supports most of the animal production in small freshwater ecosystems (Brett *et al.*, 2017). Studies performed in heavily shaded headwater ecosystems have underscored that primary production and consumer biomass have a positive relationship with light flux (Hill, Ryon & Schilling, 1995;  Kiffney, Richardson & Bull, 2003). Therefore, variation in light has been proposed as an important factor affecting the strength of food limitation. For example, even a limited decrease in light availability can strongly reduce the abundance of shredders, the biomass of fungi and the rate of litter breakdown in streams (Lagrue *et al.*, 2011). However, the relationships between light intensity and the abundance of different taxa may be complex, for instance because light flux may interact with the abundance of top predators, thus constraining the abundance at lower trophic levels (Kiffney, 2008).

Caves represent a system where local production and allochthonous inputs are easily discernible. Lack of light determines heterotrophic food webs completely dependent on allochthonous inputs (Romero, 2009;  Schneider, Christman & Fagan, 2011). When allochthonous detritus subsidises a habitat, the type of response displayed by the organisms depends on the trophic level that receives the input, on the resource features (Mammola & Isaia, 2016), and also on

the abiotic features of the habitat itself (Schneider, Christman & Fagan, 2011; Schneider, Kay & Fagan, 2010).

Although cave habitats are often reported as ideal donor-controlled habitats (Polis et al. 1997), few studies have analysed the effects of subsidies on cave communities (Schneider, Christman & Fagan, 2011) and no studies have analysed the possible subsidy effect caused by the predator trophic level. The types of trophic inputs entering cave environments are highly variable and include dead leaves and wood (Pipan *et al.*, 2008; Romero, 2009), and the faecal material deposited by bats and insects (Fagan, Lutscher & Schneider, 2007). Moreover, organisms entering caves may themselves be an input of energy for the system (Lunghi, Manenti & Ficetola, 2014; Manenti, 2014).

Salamanders have a particularly important role in small aquatic systems (Davic & Welsh, 2004). The European fire salamander (*Salamandra salamandra*) is a predator with a terrestrial adult phase and an aquatic larval phase (Krause & Caspers, 2015; Manenti, Ficetola & De Bernardi, 2009; Steinfartz *et al.*, 2006). These salamanders generally breed in streams (Denoël & Winandy, 2014), and may have significant effects on subsidy exchanges between pools and surrounding woods (Reinhardt *et al.*, 2013). The species is usually ovoviviparous, and adult salamanders feed exclusively in the terrestrial habitat, thus the biomass of laid larvae does not depend on the resources of the aquatic ecosystem where they live (Reinhardt et al, 2013). Therefore, birth and metamorphosis of larvae determine consistent bidirectional fluxes of biomass between aquatic breeding sites and the terrestrial environment (Reinhardt *et al.*, 2013). The fire salamander has highly plastic breeding strategies, and in karst areas it also actively uses cave pools as its breeding habitat (Manenti *et al.*, 2009; Manenti *et al.*, 2011). Salamanders are able to successfully develop in cave pools, but the underground environment strongly affects larval development, which in caves requires more time to reach metamorphosis than in streams ((Limongi *et al.*, 2015; Manenti *et al.*, 2011). The cave waterbodies are predator-free, therefore fire salamander larvae are the top predators in this system. Similar to what occurs in streams, larvae deposited in cave pools represent an intake of biomass from the outdoor terrestrial environments for cave pools, while metamorphosing individuals export biomass from the underground aquatic environment to the surrounding terrestrial one.

The aim of this study was to assess the factors determining the balance of biomass in cave and spring pools. In particular, we examined (1) how light intensity, together with other environmental features, drives biomass export and (2) if top predators constitute a trophic subsidy in these ecosystems. These aspects provide new insights into the role played by predators in the energy flows of small freshwater systems.

## Materials and methods

From March to August 2013, and from March to August 2014, we monitored 21 fire salamander breeding sites (12 cave pools and 9 spring pools). Sites were relatively isolated spring or cave pools (Suppl. Fig. 1). Both cave and spring pools were headwater, therefore compensatory drift (Krause & Caspers, 2015; Reinhardt *et al.*, 2013) of fire salamander larvae was impossible. In both habitats pools were fed by water from the aquifer and showed limited flow, being distant from eventual downstream running freshwater habitats. All the sites were located in Lombardy (NW Italy) in the Pre-alpine region (Fig. 1). Each site was surveyed weekly, for a total of 24 surveys per site during the same period; each site was monitored during one single year; two years were necessary to follow all the sites. In 2013 we surveyed 6 cave and 5 spring pools, while in 2014 we surveyed 6 cave and 4 spring pools.

In each site, fire salamander larvae were caught using a small dip net (mesh size: 1 mm). Catching was performed until no larvae were visible in the site, and was continued for at least 10 min after capturing the last larva. Larvae were placed in a small aquarium (20x10 cm, depth 5 cm), and the capture session was repeated 30 min later. For each larva, we recorded total length (accuracy: 1 mm) and weight (accuracy: 0.01 g). Furthermore, following the recommendation of (Eitam & Blaustein, 2002), the larvae were photographed laterally with a Casio Exilim Ex H30 camera to allow individual identification on the basis of tail colour pattern. The abundance of salamanders for each site and sampling session was estimated on the basis of removal sampling. Removal sampling is an approach to population size estimation that requires the systematic capture and removal of individuals. Population size can be estimated on the basis of the decline in catch size during the sequential sessions of capture and catch efforts. Population size was estimated using the homogeneous capture probability model proposed by Chao & Chang (1999), which provides reliable estimates of the number of individuals present.

The quantification of biomass balance was obtained by comparing the biomass of larvae deposited in the spring to the biomass of metamorphosing larvae during the period studied, which represents the biomass export from each site through salamanders (Reinhardt *et al.*, 2013). On the whole, we collected and weighed more than 1540 larvae. To quantify the larval biomass input, we first calculated the maximum number of new-born larvae laid in each site on the basis of the removal method and then multiplied the average body weight of newborns at a given site by the estimated number of new-born larvae (Reinhardt *et al.*, 2013). Fire salamander larvae stop feeding at late development stages (Reinhardt *et al.*, 2013). Therefore, to estimate biomass export we considered the weight of all the fire salamander larvae (distinguished individually on the basis of their lateral colour pattern) that reached the 3B pre-metamorphosis developmental stage (Juszcyk & Zakrzewski, 1981) at a given site (Suppl. Fig. 2). For estimating biomass export, we considered the total weight of individuals collected through removal samplings at the pre-metamorphosis stage; at the 3B stages salamander larvae have already established their aposematic coloration and their detectability is very high, thus limiting the risk of overlooking individuals. We considered as exported biomass the weight recorded for 3B larvae that were not observed in successive surveys. The sum of these weights was considered as the exported biomass at the fire salamander larvae level.

We measured both abiotic and biotic variables potentially related to the balance of biomass export at fire salamander level. At 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 measures of predation pressure (biomass of predators), intraspecific competition (abundance of salamander larvae) and an estimate of prey availability (biomass of potential prey).

Macroinvertebrate biomass was measured using pipe sampling (Dodd, 2010). Samples were collected by thrusting a 0.3 m$^2$ circular pipe sampler through the water column and about 5 cm into the sediment. Small nets (mesh size: 1 mm) were used to remove all animals from the water and the first cm of the sediment (Dodd, 2010). Net sweeps were collected until at least 10 consecutive sweeps were empty; for each site we repeated pipe sampling twice. Once captured, we weighed macroinvertebrates on a digital PESOLA balance (precision 0.01 g), by distinguishing between potential prey and predators of fire salamander larvae, on the basis of taxonomy and dimensions. As predators, we considered freshwater crayfish longer than 35 mm and dragonfly larvae longer than 20 mm (Bo *et al.*, 2011; Ghia *et al.*, 2009; Manenti, Siesa & Ficetola, 2013). Smaller macroinvertebrates were considered as prey; we did not record crayfish <35 mm. We then estimated prey and predator wet biomass for each site (g/m2).

We used linear models (LMs) to assess the factors determining the balance of biomass export, which was considered as a dependent variable: as independent variables we considered average maximum illuminance recorded during the whole period, site type (cave / spring), average water temperature, fire salamander average density, predator average biomass and prey average biomass. Salamander density and export biomass were not correlated (r = 0.20; t = 0.86, p = 0.39), thus suggesting that the two variables are essentially independent. If needed, independent variables were transformed using a logarithm (illuminance, larvae density, prey and predator biomass) to reduce skewness and improve normality. We built models representing all the possible combinations of independent variables and ranked them on the basis of corrected Akaikes' Information Criterion (AICc) (Rolls, 2011). As AICc may select overly complex models, we considered a complex model only if it showed AICc less than the AICc of all of its simpler nested models (Richards, Whittingham & Stephens, 2011). Models expressing the highest proportion of variation using the smallest number of predictors have the smallest AICc values and are considered the "best models" (Lukacs et al., 2007). We also assessed significance of variables composing the best model using a likelihood ratio test (Bolker *et al.*, 2008). We performed all the analyses in R 3.31 environment using the, nlme, car, and MuMIn packages.

## Results

We recorded fire salamander larvae metamorphosis events in all the cave and spring pools. Cave pools were generally dark, but light partially hit some of the pools (average ± SE illuminance of the most luminous part of cave pools: 54.3 ± 34 lux; range 0.00 – 400 lux), particularly in sites where the pool used for larvae deposition was close to the cave entrance. Spring pools were much more luminous, ranging from 10,100 to 45,300 lux (average ± SE: 17,171± 4,073 lux. During the study period, cave pools showed a noticeable stability in terms of constancy of the surface area, without appreciable variations. Instead, spring pools varied much more, with a maximum surface reduction of 95% (average ± SE total variation of surface in epigean sites: 13.34 % ± 4 %); a Leven's homogeneity test, performed on the surface of pools between two consecutive surveys, confirmed that the surface of spring pools underwent much stronger variations than does the surface of cave pools ($F_{1,61}$= 10.28,  p< 0.01).

No predators were detected in caves, except for one site in which we recorded two juveniles of the native crayfish *Austropotamobius pallipes* (Crustacea, Astacidae), while in spring pools predators occurred in almost all the sites ( n= 7) and were mostly composed of *Cordulegaste*r (Odonata, Cordulegasteridae) and *Aeshna* (Odonata, Aeshnidae) dragonfly larvae (maximum density 2.09 individuals/m$^2$; mean density ± ES = 1.39 ± 0.37 individuals/m$^2$).

In March, the average biomass (± SE) of newborn fire salamander larvae spring was 4.91 ± 1.93 g m-2 in caves and 19.9 ± 6.10 g m-2 in spring pools. The difference in biomass import per square meter was weakly significant ($t_{1,9}$, = 2.34,  p = 0.04). In cave pools the average biomass export was 40% of the import, while in spring pools it was 73% of the import. Maximum salamander larvae biomass in cave pools was 53.3 g m$^{-2}$, representing on average 97.3% of the total aquatic biomass of the pool. Conversely, in spring pools the maximum biomass of the larvae was 17.3 g m$^{-2}$, with salamander larvae accounting for 27.6% of the total biomass (Fig. 2).

The biomass balance was negative for 75% of cave pools, while all the spring pools had a positive balance (Table 1). According to the best-AICc model, light intensity was the most likely determinant of biomass balance, with a strong, negative relationship between biomass balance and light ($F_{1,17}$ = 20.56, P < 0.001). Although illuminance and site type were highly correlated (r= 0.83, $t_{1,18}$ = 6.4,  p< 0.0001), site type (cave vs. spring) was not included among the best AIC models, and the importance of light intensity was much higher than that of site type. In the best selected

model, larvae density was also included, but the relationship was weak and not significant ($F_{1,17} = 1.33$, $P = 0.26$). The importance of all the remaining predictors, including predator and prey biomass, was extremely low (Table 2; Table 3).


## Discussion

Fire salamander larvae constitute a major component of the animal biomass in both cave and spring pools, and are the most abundant top predators for them. Therefore, identifying the factors determining their biomass balance is pivotal to understanding the functioning of the small freshwater ecosystems in which they live. Abiotic environmental features were more important than biotic parameters in determining salamander biomass balance, as biomass production was generally highest in the sites that received more light and the most productive ones for the larvae. These results underscore that light flux is a main constraint on predator biomass; a similar pattern, but applied only to lower trophic levels, has been reported for shaded headwater streams in which primary producer and consumer biomasses are constrained by light flux which interacts with nutrient supply and predator abundance (Kiffney, 2008). Primary producers, such as periphyton, photosynthetic bacteria occurring in biofilms and other photosynthetic organisms, are strongly limited in shady environments (Chase, 2003) and several studies in shaded headwaters have shown that light flux increases primary production and consumer biomass (Hill, Ryon & Schilling, 1995; Kiffney, 2008), even though high light flux over a certain level can limit primary production through photoinhibition (Hill, Ryon & Schilling, 1995; Kiffney, Richardson & Bull, 2003; Murray, Tenhunen & Nowak, 1993). Although almost all the study sites received some light, the comparison between spring and cave pools allows contrasting sites with strong differences in light, and thus identifies the strongest effects of light.

In cave pools, the biomass balance was generally negative, with a pattern opposite to that observed in springs. Our study demonstrates that cave aquatic environments, even when close to cave entrances, are clearly less subsidising ecosystems than outdoor springs, even though some of them occur within heavily shaded forests. This may be linked to different factors. First, in headwater streams and springs, the occurrence of periphyton biofilms and other primary producers increases the availability of nutrients for the food web together with the detritus and organic matter deriving from the surrounding terrestrial habitat (Brett *et al.*, 2017). Recent studies demonstrate that the occurrence of benthic algae (like diatoms), although minor with respect to detritus, constitute high quality food sources for some of the invertebrates that are fire salamander prey (Crenier *et al.*, 2017).

Second, epigean sites may be more easily accessible to biphasic insects, the larvae of which often are detritivore or primary consumers (Koperski, 2011) and constitute the usual prey of the fire salamander larvae (Costa *et al.*, 2015). The adult females of salamanders feed in terrestrial habitats such as the forests surrounding breeding sites. Our results indicate that when salamanders enter caves to deposit their larvae, they deliver a major biomass subsidy to these habitats, and such a biomass input is much higher than the export that will return to the outdoor environment. This represents an interesting case of direct subsidizing from the top predator level. In this extreme case, top predators, although they exert strong predation pressures on consumers at lower trophic levels, constitute the major biomass input of the system, and thus have a major role in sustaining the whole food web. The importance of salamander larvae for the aquatic food web subsidy has been observed in other systems, such as in poorly productive temporary ponds in forests, where the amount of larval biomass that enters the ponds is generally higher than the export through metamorphosed individuals (Reinhardt *et al.*, 2013).

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The behaviour of the fire salamander actively entering caves to breed is a recently described phenomenon (Manenti *et al.*, 2009) that may be favoured by the fact that cave habitats in temperate regions are generally environments limited abundance of predators (Fiser, Blejec & Trontelj, 2012; Galassi *et al.*, 2009; Schneider, Christman & Fagan, 2011) and provide stable environmental conditions. However, cave environments also pose multiple limitations, and food scarcity severely limits growth rate (Limongi *et al.*, 2015). As a consequence, a particularly strong reproductive effort can be required to exploit underground freshwaters. Fire salamander females, with their breeding site choice, provide a substantial contribution to the biomass input in both spring and cave pools. By breeding, females subsidize heterotrophic systems in which cannibalism is likely to play a major role. Both in cave and spring pools, it is frequent to find different cohorts of larvae showing strong asymmetries in terms of both size and developmental stage. The smallest salamander larvae, belonging to the last laid cohorts, may represent an important trophic supply for the larger conspecifics (Limongi *et al.*, 2015; Romeo *et al.*, 2015). In the case of the study pools, most of the prey-predator energy flow likely occurs at the same trophic level, i.e. among top predators. The evolutionary adaptations allowing the exploitation of different environments by a single species may thus be driven by female site choice, and can determine a change in the direction of energy fluxes between aquatic and terrestrial environments, as also reported for temporary pools (Reinhardt *et al.*, 2013; Steinfartz, Weitere & Tautz, 2007).

Overall, our study highlights two key points. First, the exploitation of a habitat by the temporary life stages of organisms at the predator level does not necessarily translate into a depletion of the general biomass of an ecosystem; on the contrary, in poorly productive environments, it may constitute a trophic subsidy. Second, the biomass balance of predators may vary as a result of abiotic gradients that constrain population size, such as light incidence.

## Aknowledgments

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**Tables**

| ID Site | Pool type | Import (g) | Export (g) | Biomass balance (g) | Average temperature (°C) | Average maximum illuminance recorded (lux) | Average larvae density (g/m²) | Average prey biomass (g) | Average predator biomass (g/m²) |
|---------|-----------|-----------|-----------|---------------------|--------------------------|--------------------------------------------|-------------------------------|--------------------------|---------------------------------|
| G01 | Cave | 3.58 | 0 | -3.58 | 13.4 | 451 | 2.41 | 0.08 | 0 |
| G02 | Cave | 24.47 | 24.5 | 0.03 | 11.7 | 26.6 | 25.1 | 0.11 | 0 |
| G03 | Cave | 16.74 | 0 | -16.74 | 11.2 | 0.89 | 2.5 | 0 | 0 |
| G07 | Cave | 0.99 | 1.35 | 0.36 | 12.8 | 9.6 | 5.63 | 0.007 | 0 |
| G09 | Cave | 5.34 | 5.96 | 0.62 | 11 | 3.96 | 3.9 | 0.18 | 0 |
| G11 | Cave | 12.12 | 0 | -12.12 | 12.1 | 5.8 | 1.76 | 0 | 1.19 |
| G16 | Cave | 11.85 | 4.23 | -7.62 | 14.3 | 53 | 7.86 | 0.13 | 0 |
| G17A | Cave | 10.57 | 3.52 | -7.05 | 12.1 | 16.56 | 1.12 | 0.1 | 0 |
| G17B | Cave | 3.08 | 0 | -3.08 | 11.6 | 17 | 0.3 | 0.05 | 0 |
| G17C | Cave | 1.1 | 0 | -1.1 | 11.7 | 17 | 2.35 | 0.032 | 0 |
| G19 | Cave | 5.47 | 0 | -5.47 | 10.16 | 0 | 0 | 1.258 | 0 |
| G22 | Cave | 8.78 | 2.28 | -6.5 | 12.2 | 24.3 | 1.6 | 0.085 | 0 |
| Pozzaa | Spring | 56.12 | 75.46 | 19.34 | 11.91 | 18300 | 17.31 | 0.2 | 0.28 |
| Pozzab | Spring | 18.92 | 70.08 | 51.16 | 15.75 | 45300.00 | 2.41 | 0.17 | 0 |
| Pozzac | Spring | 86.93 | 6.15 | -80.78 | 11.26 | 10100 | 14.39 | 0.1 | 0 |
| Pozzad | Spring | 91.78 | 8.66 | -83.12 | 15.55 | 5300 | 46.74 | 3.26 | 1.89 |
| Pozza 1 | Spring | 1.16 | 3.2 | 2.04 | 17.22 | 16125 | 22.9 | 0.12 | 0.13 |
| Pozza2 | Spring | 9.43 | 11.18 | 1.75 | 17.31 | 14875 | 26.09 | 0.4 | 1.76 |
| Pozza3 | Spring | 14.28 | 16.62 | 2.34 | 15.2 | 9875 | 76.24 | 0.24 | 0 |
| Pozza 4 | Spring | 23.17 | 27.03 | 3.86 | 16.67 | 18666 | 34.17 | 3.19 | 2.4 |
| Pozza 5 | Spring | 3.53 | 7.11 | 3.58 | 18.36 | 16000 | 29.5 | 0.06 | 0.9 |

Table 1. Environmental features of the spring and cave pools surveyed.

| Variables in the best models | $R^2$ | AICc | Δ | Weight |
|---|---|---|---|---|
| Illuminance, larvae density | 0.56 | 155.2 | 0.00 | 0.59 |
| Illuminance | 0.41 | 157.8 | 2.53 | 0.17 |
| Larvae density, cave | 0.49 | 158.0 | 2.82 | 0.14 |
| Cave | 0.36 | 159.5 | 4.24 | 0.07 |
| Average temperature | 0.29 | 161.4 | 6.22 | 0.03 |
| Null model | 0.00 | 165.6 | 10.38 | 0.00 |
| Larvae density | 0.03 | 167.7 | 12.47 | 0.00 |

Table 2. Best models with AICc weight > 0.01 illustrating the relationship between biomass export and the environmental variables of the cave and spring pools.

| Variable | w | $B$ | ± SE |
|---|---|---|---|
| Illuminance | 0.76 | -7.25 | 1.66 |
| Larvae density | 0.73 | 13.75 | 0.19 |
| Cave | 0.21 | 17.01 | 0.00 |
| Averagetemperature | 0.03 | -5.20 | 0.00 |

Table 3. Model-averaged parameters of environmental variables influencing the biomass balance. w: cumulative AICc weight of the variable; $B$: averaged regression coefficient, with ± Standard Error.

Fig. 1. Study area (map shows Italian political borders). Triangles show locations of cave pools; circles show locations of spring pools. Some of the symbols are superimposed owing to geographic proximity.
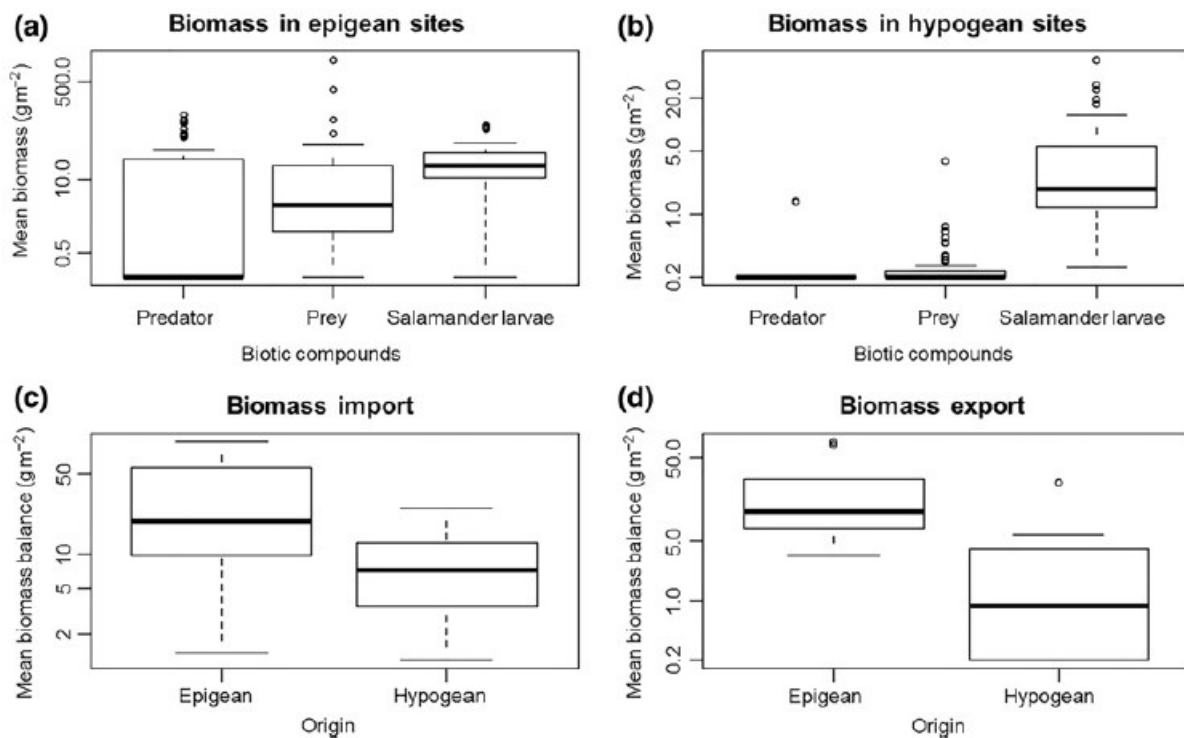


Fig. 2. Box-whisker plots of biomass levels recorded in spring and cave pools. The bold bar represents the median; upper and lower box bars represent the upper and lower quartile respectively; whiskers represent the maximum values. A= comparison between the mean biomass of salamander larvae and their predators and prey in epigean sites; B = comparison between the mean biomass of salamander larvae and their predators and prey in cave pools; C comparison of salamander larvae biomass import between spring and cave pools; D = comparison of salamander larvae biomass export between spring and cave pools.

Supplementary Fig. 1. Examples of cave and spring pools. Acronyms refer to the sites ID reported

    in Table 1. G13 and G19 are examples of cave pools; "Pozza 3" and "Pozza 4" are examples

    of spring pools.


Supplementary Fig. 2. Fire salamander larvae. A = newborn larva lateral view. B = newborn larva

    dorsal view. C = larva at the 3B stage, lateral view. D = larva at the 3B stage, dorsal view.