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PRIMARY RESEARCH PAPER

Understanding benefits and risks of exploiting spring habitats by subterranean animals: insights from a mark-recapture study on the olm (*Proteus anguinus*)

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Abstract The olm *(Proteus anguinus*) is an iconic predator in underground ecosystems, but it also exploits localized spring habitats, characterized by higher trophic abundance and predation risk. This study analyzes the advantages and disadvantages of using springs and caves, with particular attention to population variations. In the classical karst system (NE-Italy), we compared food availability and predator occurrence in 40 springs and 8 caves and monitored 76 *P. anguinus* individuals through capture-\mark-recapture (CMR) surveys. Individuals captured in springs had a significantly higher Scaled Mass index (SMI) compared to those from caves, with no significant differences in the occurrence of injuries. The greater trophic abundance and diversity in springs, combined with site fidelity, suggests that karst spring ecotones may represent critical foraging areas for stygobionts, such as animals specialized to inhabit groundwater. The results confirm the ecological importance of these karstic springs habitats, which support high biodiversity, species interactions, and serve as key points for studying the adaptive responses of *Proteus anguinus* and other subterranean species.

Keywords Groundwater · Ecotone · Resurgence · Amphibians · Cave · Plankton · Macrobenthos

Introduction

Assessing which factors allow species and populations to exploit habitats that are marginal to their

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distribution and ecology can provide useful information on pressures promoting adaptation (Kawecki 2008). Marginal habitats often constitute an ecotone, such as an area of (more or less steep) transition between ecosystems or communities that can occur at different spatial scales and include both natural boundaries and even human-generated borders (Scheiner 2023). The ability to respond to the unique conditions that ecotones offer, as opposed to core habitats, can play a critical role in the range expansion of a species, in the emergence of distinct genotypes and the development of varied phenotypes through phenotypic plasticity. Populations residing in ecotones may, thus, exploit different ecological niches than those occupied by populations in core habitats, potentially leading to the evolution of specific adaptations (Kawecki 2000).

Among ecotones, springs are particularly favorable to study how pressures can drive the emergence of different patterns from those that characterize a certain species in its core environment (Cantonati et al. 2020; Manenti et al. 2023b). Springs are ecotones between groundwater and surface freshwater habitats, characterized by a complex interplay of the features of both subterranean and epigean environments (Cantonati et al. 2022, 2006). The transition between surface and subterranean habitats is significantly influenced by the morphological characteristics of the spring and can exhibit temporal variability based on daylight conditions (Williams 1991; White 2019; Pieri et al. 2007; Manenti et al. 2023a). Some springs exhibit a sudden shift from subterranean environments to the surface, whereas others, such as those where a subterranean stream flows outward from natural caves may represent more extensive ecotonal zones (Manenti et al. 2013; Fansa Saleh et al. 2021; Gil-Meseguer et al. 2023; Khardi et al. 2023). The boundary between subterranean and surface environments can become particularly pronounced during daylight hours, as it is distinctly marked by solar illumination. Beyond sunlight, several characteristics differentiate the subterranean and surface environments within a spring, even over the short distances (a few meters or even centimeters) that can characterize this sudden interface. These characteristics include the availability of trophic resources, the density of potential predators, and physical/chemical features (Culver and Pipan 2011, 2014; Barzaghi et al. 2017; von Fumetti et al. 2017). The complex interactions between these biotic and abiotic characteristics thus create dynamic habitats that are influenced by the contrasting conditions of adjacent environments (Manenti et al. 2023b). Springs and adjacent surface habitats can be the boundary habitats for several animal species normally considered as strictly adapted to groundwater (Dole-Olivier et al. 2005; Evtimova et al. 2009; Fenolio et al. 2017; Balestra et al. 2024). These animals are defined as stygobionts (meaning inhabitants of the Styx, the subterranean river of Greek mythology) and often show traits such as absence of eyes, depigmentation, and low metabolic requirements (Romero 1985, 2020; Romero and Green 2005). Although cases of accidental drifting from groundwater can be relatively common (Malard et al. 2009, 2002), multiple stygobiont *taxa* actively exploit springs and surface environments (Manenti and Barzaghi 2021; Manenti et al. 2025). Still, we have incomplete knowledge of the ecological advantages and disadvantages experienced by stygobiont taxa exploiting the ecotone with the surface (Biró et al. 2024; Premate et al. 2024). Stygobionts are organisms that inhabit groundwater and typically exhibit marked adaptations to such subterranean environments The olm (*Proteus anguinus* Laurenti 1768) is a notable stygobiont (Friedrich 2013; Aljančič 2019) with multiple, enigmatic reports of individuals outside of their typical underground habitats (Manenti et al. 2024a). Except for a subspecies confined to a few springs in Southern Slovenia, the olm is a depigmented, blind salamander recognized as a specialist of groundwater environments (Balázs et al. 2020b); it is the only cave-dwelling vertebrate species in Europe and the first cave animal ever described and it shows obligatory paedomorphosis (Bonett et al. 2022). Recent genetic studies suggest an intricate evolutionary history, with nine divergent lineages that separated between 17 and 4 million years ago, probably following micro-tectonic and climatic changes that occurred in the range of the species, and a complex pattern of transitions between surface and the cave environment (Recknagel et al. 2024). *Proteus anguinus* is unique among amphibians in the Palearctic. This species exhibits several non-visual sensory adaptations, including magnetic field sensitivity (Schlegel 1997, 2008; Schlegel and Bulog 1997), alongside other traits associated with subterranean life, such as extended longevity, low metabolic rates, and tolerance to low levels of dissolved oxygen (Vandel and Durand 1970; Mali et al. 2013; Beale

et al. 2016). Breeding events are infrequent, and only known from animals kept in captivity (Guillaume 2000; Aljančič 2019). Within its range, *P. anguinus* is considered the apex predator of groundwater ecosystems (Uiblein et al. 1992; Manenti et al. 2020). Nonetheless, much of the available behavioral, biological, and ecological information regarding this species is derived from individuals under laboratory conditions. Field observations of *P. anguinus* are largely limited to gray literature, with few formal studies conducted to date (Balázs et al. 2015b; Vörös et al. 2017). Recent field studies revealed that olms can regularly exploit surface environments (Premate et al. 2022; Manenti et al. 2024a, 2025), where they can perform key activities such as feeding, with a potential inclination to prey upon terrestrial earthworms (Manenti et al. 2024a). Within surface environments, *P. anguinus* is associated to low flowing springs that form small pond-like habitat when the water level rises, to springs subject to flooding (i.e., sometimes flooding terrestrial areas) and to temporary springs (Manenti et al. 2025). The resources and pressures (like predation risk) found in spring habitats may influence the phenotype of olms differently than caves, which are characterized by stable temperatures but limited food.

This study aims to investigate the phenotypic differences between *Proteus anguinus* populations which individuals can regularly visit these contrasting environments, assessing advantages and disadvantages that can be related to phenotypic features. First, we estimated the amount of trophic resources available in caves and springs. Second, we also estimated through repeated field sampling the number of two fish species, the pike (*Esox cisalpinus* Bianco and Delmastro 2011) and the bullhead (*Cottus gobio* Linnaeus 1758), which can be predators of *P. anguinus* adults and juveniles, respectively.

We predict that, compared to caves, springs are resource-rich environments. If this prediction is confirmed, stygobionts could venture into springs because of the higher food availability, despite the higher predation risk they face in these environments. Although commonly hypothesized, the differences in trophic and prey content between springs and caves have rarely been studied in a study system that meets the needs of stygobionts. We then used a mark-recapture study to test whether individuals are faithful to the environment (cave or spring) in which they were recorded. By calculating the Scaled Mass Index (SMI) of each individual, we assessed whether the body mass of individuals exploiting caves differed from those using springs. The SMI provides insights into the energy reserves of salamanders, and can reveal the effect of differences of food availability (Peig and Green 2009; Ficetola et al. 2019). As springs are hypothesized to provide more trophic resources than caves, we predict higher SMI values in individuals collected in springs. Fourth, we compared the rate of injuries and malformities between cave and spring populations. If predation risk is higher in springs, we predict a higher number of individuals with injuries and malformations in such environments. Our analyses reveal the complex trade-offs experienced by cave animals exploiting the ecotones between underground and surface environments.

Material and methods

Study area

The study area (Fig. 1) is situated between the districts of Gorizia and Trieste (northeast Italy) in the north-western part of the Classical Karst. The Classical Karst is a limestone plateau extending northwest–southeast in the northernmost sector of the Eastern Adriatic karst, bounded by the lower course of the Timavo River (south), the Isonzo–Soča River (north), the Adriatic Sea (west), and the Vipava Valley (east). Here we sampled springs and caves that are mostly interconnected within a system of karst lakes comprising Lake Doberdò, Lake Pietrarossa, Lake Sablici and Lake Mucille (Zini et al. 2022). These depressions are situated at altitudes ranging from 1 and 4 m a.s.l. During floods, water input rapidly increases, exceeding sinkhole drainage capacity and causing abrupt rises in water levels (Zini et al. 2022). As an example, the water level of the Doberdò Lake, which is on average 4.78 m a.s.l., can usually reach 8 m a.s.l. during floods with peaks of 11 m a.s.l. (Zini et al. 2022).

Food availability and predator occurrence assessment

The assessment of food availability was carried out during the spring season, when the water level is usually high, from 2 to 5 May, 2022, allowing comparisons within these contrasting aquatic environments.

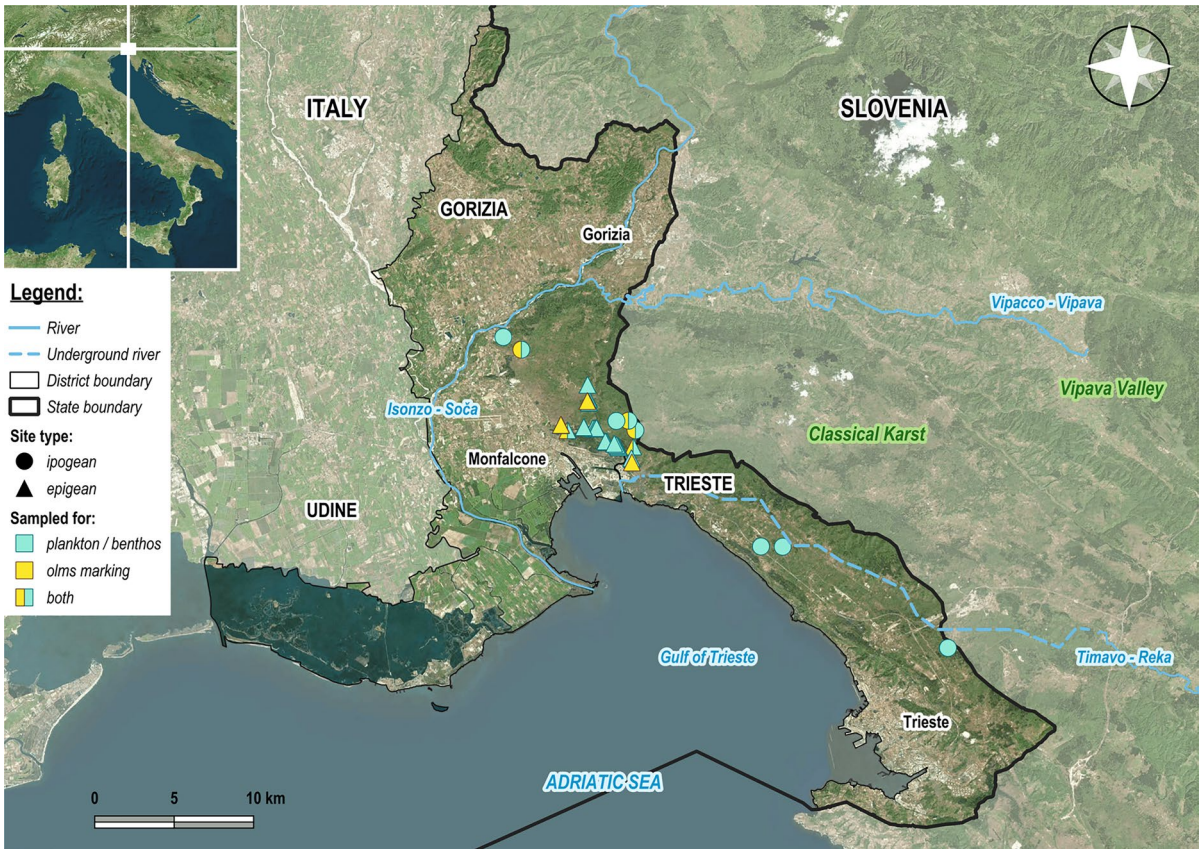

Fig. 1 Map of the study area. We introduced an error in the locations of *Proteus anguinus* occurrence to limit the risk of poaching (Lunghi et al. 2019a). Moreover, since 2024 all the surface sites not in private property where *P. anguinus* lives are controlled by video surveillance

A total of 40 springs and 8 caves were selected for this activity and underwent extensive plankton and benthos measurements. Sampling was carried out during daylight hours. Plankton sampling methods were based on standard protocols (Sameoto et al. 2000). We used simple nets to cut costs without losing effectiveness. Plankton collection was performed by filtering 10 l of water through white handkerchief of cotton fabric. Loss of organisms through meshes is an important issue when sampling zooplankton (Sameoto et al. 2000). To prevent loss of the effectivity of the mesh as well as cross-contaminations, we used each handkerchief only once and then threw it away. After filtering, the handkerchief was rinsed in a plastic container with 50 ml of water to resuspend what was captured on the mesh. The final suspension was put into a centrifuge tube, marked with the name of the spring, date and time of day, and then stored in a fridge at 4 °C until being analyzed under a Leica M165 FC stereoscope. We evaluated different *taxa* using light microscopy (Table 1).

Following the plankton sampling, we proceeded with the benthos collection using the disturbance-removal sampling method: a 20 × 20 cm flexible metal square was placed onto the stream bed of the selected springs and caves, followed by dredging the top layer of soil by hand and capturing the macroinvertebrates

Table 1 List of the plankton taxa sampled and numbered in both caves and springs

Microplankton	Mesoplankton	Macroplankton
Ciliata	Rotifera	Copepoda
Gastrotricha	Hydrachnida	
	Ostracoda	
	Nauplius	

moving in the water column using a handheld fish net (15 × 10 cm wide, 1 mm mesh size). The procedure was conducted over a period of 90 s each time and was designed to sample the streambed epifauna and the shallow burrowing fauna. The number of squares analyzed for each site was proportional to the area of the spring/cave: one square per 2 m^2, with a maximum of 10 squares for two sampling sites in the Trebiciano cave. Average area of sampling was 1.14 m^2 (SE = 0.16). The animals that were captured this way were then placed into small plastic containers, weighed, classified into broad taxa, and counted. After all measurements were completed, the animals were released. We based our protocol on reports by Holme (1964) and on standard methods for macrobenthos diversity and abundance assessment (Ghetti 1997).

For predators' occurrence assessment, we sampled multiple times (both during day and during night) the same sites used for food availability assessment from February 2022 to May 2023 and we visually recorded the occurrence of predatory fish species like pikes (*Esox cisalpinus*) and bullheads (genus *Cottus*). According to local reports (Paradisi et al. 2021), these two fish species occupy top-predator positions in the trophic webs of the study area. We hypothesized that they may prey on olms when the latter emerge from groundwater into springs.

We also assessed whether the sites monitored dried during the study period, using a multi-method approach with various data collection techniques to determine if water was present or absent at the different sites. Due to methodological differences, a broad measure of drying was employed, indicating if a site dried at least once during the study rather than precise metrics like frequency or duration. Camera traps were used at five springs to monitor hydrological connectivity, while the subterranean water level was tracked in the Trebiciano Cave (where it is possible to access the Timavo River), serving as a proxy for groundwater fluctuations during droughts. Field surveys of springs and predator fish occurrence were conducted, averaging 10.3 visits over 16 months, with frequency varying based on water presence. Springs without camera traps were checked approximately every 30–40 days, and cave surveys occurred 4 to 11 times, including during the lowest water levels in May 2022. By combining these data sources, the study provided insights into drying patterns across the sites.

Surveys of *Proteus anguinus*

From October 2022 to May 2023, we surveyed eight springs and three caves within the same area to capture and mark *Proteus anguinus* individuals. The minimum distance between the sampled springs and the sampled caves is 1.63 km, while between adjacent springs of 15 m. In one cave and three springs, we performed four to six samplings of *P. anguinus* individuals, while in the other caves we repeated the samplings only twice because of difficulties in collecting the observed individuals. The springs that we investigated more than twice are the sites "154p", "Maneggio" and "Sab_1" (for details see (Manenti et al. 2025)). The first two sites show remarkable variations in terms of hydroperiods, while the third is more stable. The cave is named "Grotta del Proteo" and is accessible with permission from a private house in the municipality of Sagrado. It also shows strong variations in the water level; during dry periods only a very small siphon exists, while when the water level raises, two temporary pools can be reached by *P. anguinus* individuals.

Sampling in the springs was carried out at night, from 8 p.m. to 2 a.m., while sampling in the cave occurred generally between noon and 4 p.m. During samplings, we approached the sites using lamplights and employed a dip-net to capture the individuals. It is noteworthy that *P. anguinus*, despite being blind, exhibits relatively rapid behaviors to seek refuge in the aquifer when disturbed, even while active in surface springs (Manenti et al. 2025). Consequently, the observed number of active individuals frequently exceeded the number captured. Each collected individual was weighed, photographed dorsally on millimeter paper, and subsequently marked using Visible Implant Elastomers (VIEs; Northwest Marine Technology, Inc., Shaw Island, Washington). VIEs are an effective and durable method for marking amphibians (Lunghi et al. 2019b, 2022; Balázs et al. 2020a). The protocol developed by Balázs et al. (2015b) was followed, wherein VIEs were gently injected at various locations beneath the tail-fin skin of each individual using a standard 29-gauge needle. The entire marking process for a single salamander was completed in < 3 min., and all individuals were released at the sites of capture soon after sampling. Additionally, we noted and photographed the occurrence of possible

body anomalies (Fig. 2). The anomalies observed in individuals have been categorized as follows:

-injuries: these are anomalies that could be attributed to non-fatal predation. These lesions include bite marks, the absence of body parts (such as limbs or digits), or regenerating limbs.

-malformations: these anomalies are less likely to be associated with predation. Malformations include irregular tail profiles, an increased number of digits beyond the norm, atrophied gills, and crooked body parts (such as the neck or spine).

After the first sampling, at each survey we recognized the marked individuals, and we marked those not previously collected. We also tentatively assessed sex of individuals based on the cloaca shape, but as this can be misleading (Holtze et al. 2017) we did not consider sex for further analyses.

Mark-recapture data were used to assess the repeated use of spring and cave sites by the individuals. Dorsal pictures were used to measure the total length and the SVL length following the method of Lunghi et al. (2020). For each salamander, we used total length and weight to calculate for each individual the SMI, (Peig and Green 2009) which represents a good proxy of the relative size of energy reserves and other body components for salamanders (Lunghi et al. 2018b). The formula used to calculate the SMI is exactly the same proposed by Peig and Green (2009), such as.

$SMI = Mi\left[\frac{L0}{Li}\right]^{bSMA}$, where L is the linear body length of the individual i, M is the body mass of the individual i, bSMA is a scaling exponent calculated by a standardized major axis (SMA) regression of M

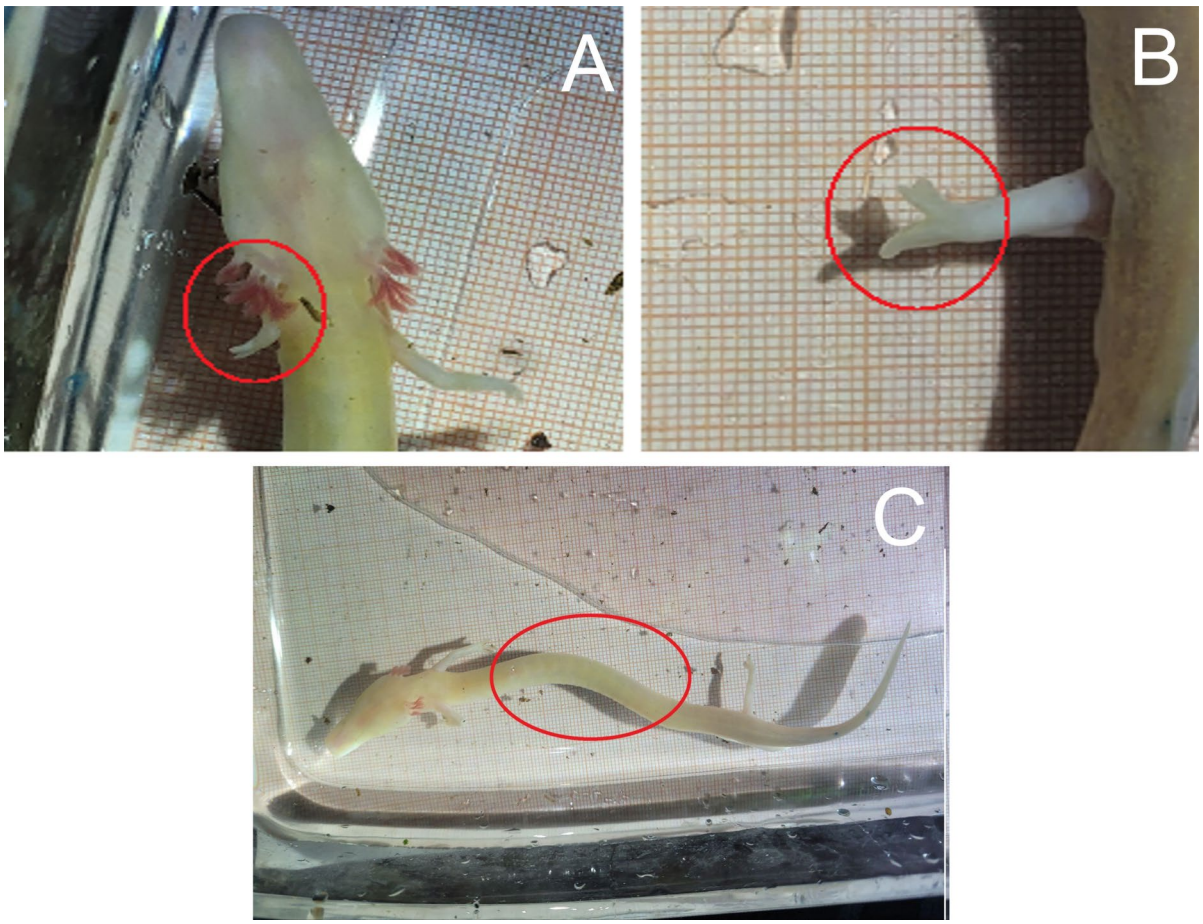

Fig. 2 Examples of malformities and injuries recorded in *Proteus anguinus* individuals (evidenced by the red circle). **A** regenerating limb; **B** bitten finger; **C** crooked spine

on L and L0 is a value of L (e.g., the arithmetic mean value for the study site).

Statistical analyses

To assess differences of available trophic resources and of predator occurrence between springs and caves, we used a multivariate Bayesian generalized linear model (GLM) with negative binomial error distribution The dependent variables were the abundance (number of individuals) of micro-, meso- and macroplankton, the number of macroinvertebrate taxa and the number of predatory fish species recorded at least in one survey during the sampling period in each site. The independent variables were the origin of the samples (cave or spring) and hydroperiod of the site (i.e., if it dried or not at least one time between February 2022 to May 2023).

To test whether olms from springs and caves show different mass index (SMI value), we built a linear mixed model (LMM) using the SMI value of each individual as the dependent variable. The type of habitat was used as a fixed factor and the identity of the sites of occurrence as the random factor. We assessed fixed factor significance using a likelihood ratio test (Bolker et al. 2008). SMI values were log-transformed to improve normality.

To assess differences between springs and caves in the occurrence of malformations, we used two generalized linear mixed models (GLMM) with binomial error distribution. In one model the dependent variable was the occurrence of at least one injury and the other the occurrence of at least one malformation; as a fixed factor we used the type of habitat. The identity of the sites of occurrence was the random factor. We assessed fixed factors significance using a likelihood ratio test. All the analyses were performed in R environment (R version 4.1.1) using the packages brms, lme4, lmerTest, and car.

Results

On average, spring sites showed generally more plankton, higher diversity of macrobenthos taxa and higher occurrence of predatory fish (Table 2). Multivariate Bayesian GLM showed strong differences in trophic resources availability between caves and springs while there were no differences between temporary and perennial springs (Table 3). In springs there are more macroplankton, more richness of potential macroinvertebrate prey taxa and higher occurrence of predatory fish (Table 3, Fig. 3). No significant differences occurred between springs and caves for the abundance of mesoplankton and microplankton organisms.

During our samplings, we documented 152 encounters (107 in springs, 45 in caves) with individuals of *Proteus anguinus*. We successfully captured 76 of the encountered individuals (21 captures from caves and 55 from springs; total: 68 unique individuals captured). The total lengths of the individuals collected varied from 11.6 cm to 26.5 cm. A total of 64 individuals were marked, while 4 juveniles were deemed too small for marking (they were below 15 cm and 5 g). Eight captures involved previously marked individuals; one individual was recaptured twice following initial marking. Two individuals were marked and recaptured in the same cave and five were marked and recaptured in the same spring. All recaptured individuals were found at the site of their initial capture. In springs,

Table 2 Average ± SE abundance (i.e. number of individuals quantified according to the different methodologies used for each group) of micro-, meso-, and macro- plankton and of taxa of macrobenthos and predatory fish species distinguished both between caves and springs and between perennial and temporary sites during the study period

	Cave		Spring		Perennial		Temporary	
	Average	SE	Average	SE	Average	SE	Average	SE
Abundance microplankton	4.1	3.08	19.26	15.64	27.24	15.28	1.22	0.78
Abundance macroplankton	0.2	0.20	5.31	1.83	5.97	1.81	2.22	0.78
Abundance mesoplankton	1.2	1.09	3.71	1.16	3.86	1.12	3.11	1.70
N taxa macroinvertebrates	1.2	0.29	4.24	0.38	3.50	0.35	4.22	0.78
N predatory fish species	0	0.00	0.71	0.12	0.79	0.12	0.22	0.15

Table 3 Results of multivariate Bayesian generalized linear model (GLM) to assess differences in abundance of trophic resources and predator occurrence between springs and caves and between perennial and temporary sites

Dependent variable	Spring		Perennial	
	B	*CI 95%*	*B*	*CI 95%*
Abundance microplankton	0.04	− 5.15 /3.58	1.79	− 3.85 /5.60
Abundance macroplankton	**3.62**	**1.55 /5.84**	0.81	− 1.05 /2.36
Abundance mesoplankton	1.12	− 0.93 /2.81	0.01	− 2.14 /1.69
N taxa macroinvertebrates	**1.27**	**0.69/1.92**	− 0.23	− 0.63 /0.18
N predatory fish species	**16.82**	**2.71/50.66**	1.2	− 0.18 /3.10

In bold are evidenced the significant relationships

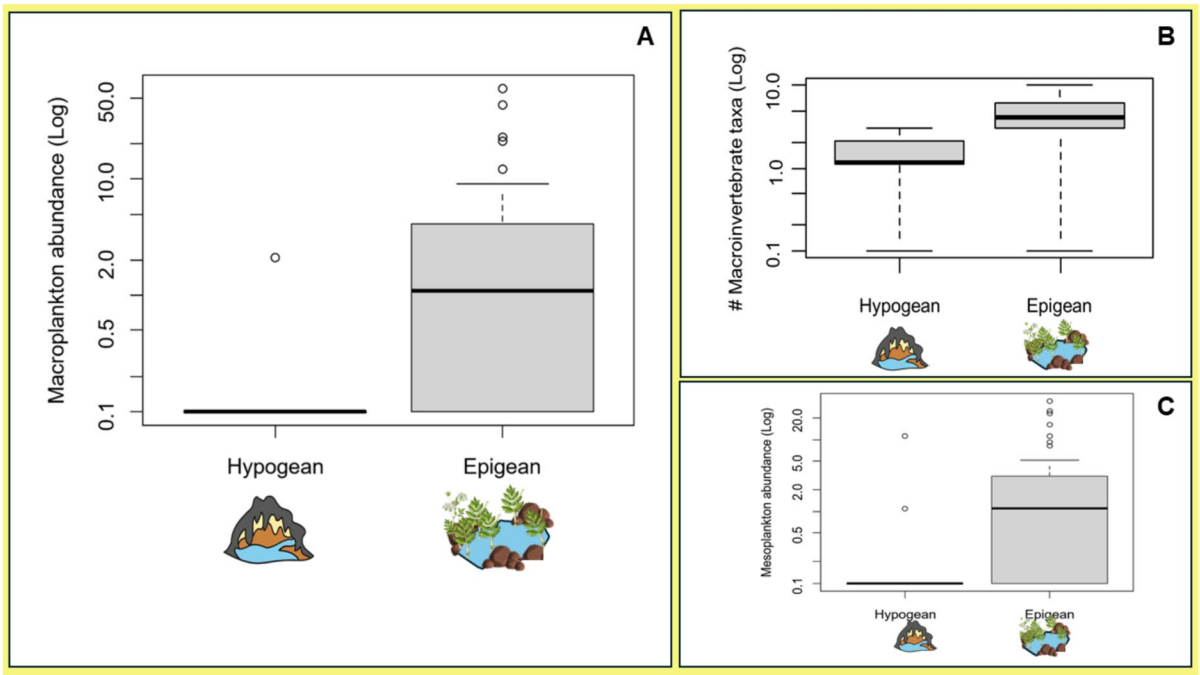

Fig. 3 Differences of trophic resources between caves and springs. **A** Boxplot showing the macroplankton abundance in subterranean (hypogean) and spring (epigean) habitats. **B** Boxplot showing the mesoplankton abundance in subterranean (hypogean) and spring (epigean) habitats. **C** Boxplot showing the number of macroinvertebrate taxa in subterranean (hypogean) and spring (epigean) habitats. The grey boxes represent 95% confidence interval, whiskers represent upper and lower extremes, and the black line represents the median

the collected olms weighed on average (± SE) 15.08 (± 1.05) g and measured 20.5 (± 0.46) cm; in caves they weighed 11.16 (± 1.20) g and measured 19.39 (± 0.74) cm. SMI values ranged between 7.7 and 23.7. Olms from springs showed significantly higher SMI ($F_{1,52} = 49.56$; $P < 0.001$, Fig. 3).

On the whole sample, 25% of the individuals collected showed an injury or a malformity. "Maneggio" and "Sab1" springs showed 37% and 14% of the individuals collected with some anomaly.

Regarding injuries, we identified 2 individuals with at least one injury within the cave environment, and 8 individuals in spring habitats. About malformations, 4 individuals with at least a malformation occurred in springs and 3 in caves. Considering just individuals

with at least an injury or a malformity, those collected in surface environments were characterized for the 69.23% by injuries probably related to predation events or snaps and for the 30.77% by other malformities. Injuries included damages at the tail and at the limbs, while malformities were mostly related to gills deformities and, in one case, to malformity of the spinal column (Fig. 2C). GLMM tests did not reveal any effect of the environment of collection on the probability to be affected by injuries ($\chi^2_{1,61}$ 0.56; P = 0.45) nor on the probability to be affected by malformities ($\chi^2_{1,61}$ = 0.61; P = 0.43).

Discussion

The results of the CMR study performed on *Proteus anguinus* from caves and springs allow us to point out new basepoints for understanding the biology and ecology of this iconic species at the interface between groundwater and surface freshwater. *Proteus anguinus* can regularly exploit transition habitats with surface, where it performs key activities, such as feeding (Manenti et al. 2024a). Our results reveal that some individuals repeatedly exploit springs and that exploiting springs can provide some advantages, particularly related to food availability (Fig. 4).

The assessment of trophic resource availability revealed significant differences between springs and caves, confirming that springs can be advantageous habitats for stygobionts to forage (Manenti et al. 2024b, 2025). The availability of macroplankton and diversity of macroinvertebrates was higher in springs than in caves. Even though no field studies on the diet of the olm have been published, both groups could be potential prey for olm larvae and adults (Lanza et al. 2009). Although macroinvertebrate diversity differed significantly among sites, a preliminary analysis indicated no significant difference in biomass. This is primarily due to the relatively high abundance of a few *taxa* at certain sites. As an example the abundance of *Troglocaris planinensis* Birstein, 1948 shrimps, which is a prey of *P. anguinus* (Jugovic et al. 2010) was more than 12 individuals per square meter in the cave "Grotta Ferrovia 214". Moreover, in springs it has been observed that olms prey on terrestrial invertebrates such as earthworms, that live in soil layers that are reached by water when the water table of karst polje raises (Manenti et al. 2024a). Also, predator occurrence differed between caves and springs, with the latter used by both pikes (*Esox cisalpinus*) and bullheads (*Cottus* sp.). They occurred mostly in perennial sites (Table 2). Pike was detected in 39% of the springs, while bullhead in the 30%. It is possible that the use by *P. anguinus* of mostly temporary spring sites reported in previous work (Manenti et al. 2025) can be linked to the fact that fish and other aquatic predators occupy perennial springs.

Considering the repeated use of the same site, the fact that four *P. anguinus* individuals have been recaptured once and one individual twice in the same spring demonstrates that the exploitation of surface environments by this stygobiont species can be an active and repeated pattern. In general, repeated

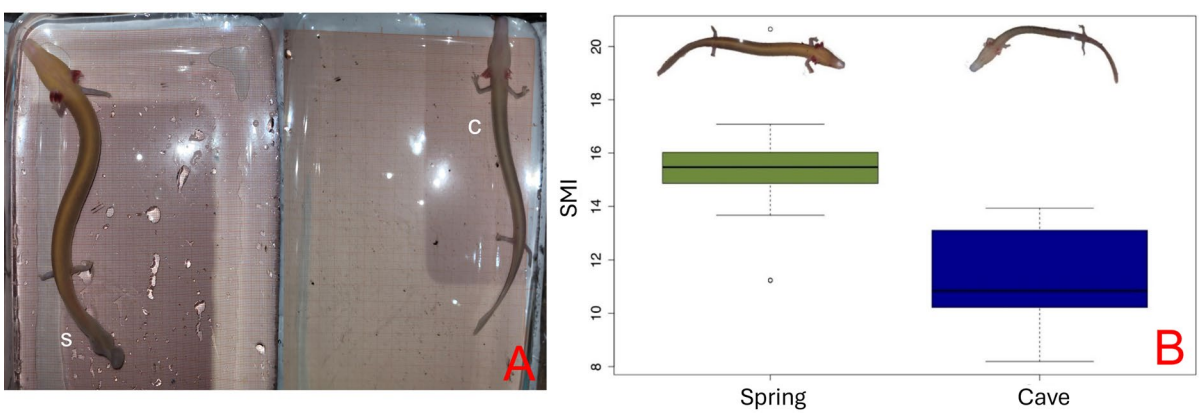

Fig. 4 Differences between olms collected in springs and olms collected in caves. **A** examples of individuals collected in springs (s) and in caves (c). **B** boxplot showing SMI index differences in olms from springs (green) and caves (blue)

use of the same site has been reported for terrestrial and semi-aquatic salamanders (Schulte et al. 2007; Caspers and Steinfartz 2011; Winandy et al. 2017; Lunghi et al. 2018a). Studies on aquatic species are rare but specifically involve the olm (Balázs et al. 2015a, 2020a). They show that fidelity can be quite strong with very limited movements occurring even across years (Balázs et al. 2020a). Our surveys were performed at night; however, recent observations indicate that *P. anguinus* can also exploit springs during daytime (Manenti et al. 2024a). Consequently, further research is warranted to elucidate the species diel patterns in both springs and caves, as well as to identify microhabitat features that may affect the extent and duration of springs exploitation. Given the extended lifespan of *P. anguinus*, future surveys conducted at the same locations, as well as expanded sampling across additional subterranean and surface sites, will enhance prospects for a comprehensive understanding of SMI variation and habitat utilization over the long term. Currently, we do not know of any springs adjacent to caves (see Methods), but recent surveys allowed us to discover two caves that are around 100 m far from the "154p" site that could be surveyed in the future. Few long-term CMR studies have been performed on paedomorphic amphibians (Denoël et al. 2007); repeated investigations of this kind could further elucidate patterns of site fidelity and body condition dynamics over time.

Compared to the submerged caves where *P. anguinus* underground distribution is usually studied (Balázs et al. 2015b; Koller Šaric et al. 2019), most of the sites considered in this study, including one of the caves, are rather instable environments. During the study period, our sampling sites experienced alternating phases of complete desiccation (four springs and the cave with the highest number of olms captured) and flooding (six springs). In some cases, the water level reached up to four meters above the spring mouth. As a result, the olms were compelled to navigate these fluctuations in water levels, traversing the ecotone between groundwater and surface habitats. The limited number of recaptures of tagged individuals may be attributable to the infrequency of their movements or may indicate a relatively high population density (Zakšek et al. 2018). The variability in the hydroperiod of springs also influenced the feasibility of conducting multiple surveys at certain locations. To improve our understanding of the dispersal capabilities and home range dynamics of *Proteus anguinus*, it would be beneficial to increase the number of surveys and the number of sites included in CMR sampling in the study area. It would also be useful to study interconnected systems of both surface and accessible deep groundwater sites from various regions. This would provide a more comprehensive perspective on the species' dispersion abilities and variations in individuals' home ranges.

The marking of *P. anguinus* individuals allowed us to make interesting comparisons between individuals collected in the cave and in springs, without the risk of including the same salamander more than once. By considering variation of SMI and injuries/malformations in the two different environments, we were able to account for both potential advantages (linked to the richer trophic content of springs) and disadvantages (related to the higher risk of predation of springs) of surface exploitation by a typical subterranean species. There were strong differences in SMI showing that the body condition of olms able to move across the ecotone and exploit springs was significantly better than that of olms collected in caves. However, the use of one of the monitored caves (where sampling was only possible when the water level reached two normally dry chambers) appeared to be associated with foraging activity, as salamanders were found feeding on earthworms. This could mean that foraging opportunities may be even more limited for individuals that remain at lower levels of the karst water table. The possibility to exploit ecotones, at least during some periods of the year, thus seems to provide clear advantages to olms. At the same time, the incidence of injuries and malformations did not vary significantly between caves and springs, suggesting, at least in our study system, that they can provide comparable disadvantages. This was an unexpected results that apparently contradicts the initial hypothesis of the caves as a safer environment (Melotto et al. 2019). Surprisingly 2 individuals of *P. anguinus* with injuries were collected in cave habitat, even if we did not record any potential predator. Intraspecific aggressiveness or damages linked to the use of interstitial habitats cannot be excluded. In general, the number of injuries and malformations recorded was low, likely affecting the robustness of the comparisons performed.

To finally disentangle this, studies on fish communities found in these springs may be necessary

and could be linked to investigations of fish effects on surficial paedomorphic amphibians (Winandy and Denoël 2013; Bernabo and Brunelli 2019). As an example, in ponds, the occurrence of fish can strongly affect the dynamics of habitat use for both feeding and breeding (Kloskowski 2009; Denoel and Winandy 2015) with strong predation and impact on the survival of paedomorphic newt populations (Toli et al. 2020). Understanding the predation risk posed by epigean fish in ecotones and groundwater habitats, as well as assessing how paedomorphic salamanders can perceive and respond to this threat, is of critical importance for elucidating their evolutionary trajectories. Nevertheless, the question on the duration of spring habitat use by the single individuals of *P. anguinus* and the extent of their return to the groundwater habitat, remains open. While it is reasonable to assume that in temporary springs those salamanders follow the fluctuations of the water level between underground and surface environments, the use of ecotonal zones in perennial springs (such as "Sab1") appears to be relatively stable.

Ecotones, including springs, often support a high degree of biodiversity and species interactions (Di Sabatino et al. 2008; Di Lorenzo et al. 2018), serving as important zones for evolutionary change (Howarth 1972). The selective pressures experienced in these transitional habitats, such as variations in resource availability, predation, and microclimatic factors, can drive adaptive responses in resident species. By comparing populations or sub-populations linked, at least for some periods, to the surface habitat and those inhabiting deeper areas it will be possible to gain further information on the processes driving adaptation to new adjacent ecosystems (Copilas-Ciocianu et al. 2017; Balázs et al. 2021).

Our study lays the groundwork for an in-depth exploration of two primary hypotheses that remain unresolved and are not mutually exclusive. First, the connections between subterranean ecosystems and surface environments may be stronger than previously recognized, suggesting that the ecotone between groundwater and surface (including both aquatic and terrestrial landscapes) is more pronounced than our perceptions indicate. The intrinsic links between these environments, particularly concerning energy fluxes and environmental stimuli, may not differ significantly, at least not to the extent that human observers can readily detect, sustaining the importance of the concept of shallow subterranean habitats (SSH) (Culver et al. 2012; Pipan and Culver 2013; Gilbert et al. 2018; Keany et al. 2019; Mammola et al. 2019). SSH could be seen as similar to surface environments and distinct to deeper subterranean systems not directly relying on energy deriving from the surface (Sarbu et al. 2018). At the same time, the exploitation of SSH should be related to the numerous and still ongoing investigations regarding how adaptations to the unique characteristics of hypogean habitats are developed and maintained (Ferreira et al. 2011; Griebler et al. 2014; Lunghi and Zhao 2020; Mammola et al. 2020, 2022; Bancila et al. 2021).

On another note, we can also take into consideration the hypothesis that the differing pressures and advantages presented by groundwater versus surface waters could drive, at least in some conditions, significant adaptations and differentiation among species residing in springs and caves. Even with ongoing gene-flow, morphological and behavioral differences can be promoted between adjacent environments (Storfer et al. 1999; Velo-Anton et al. 2012; Stern et al. 2017; Manenti et al. 2023c). Thus, a pressing question that requires further examination is whether individuals of *Proteus anguinus*—and potentially other stygobiont species—are capable of perceiving the distinct features of subterranean and surface habitats. Moreover, can these organisms respond to stimuli typically associated with surface environments, such as predator and prey cues emanating from customary surface species? This critical line of inquiry warrants thorough further investigation of stygobiont species inhabiting both subterranean and surface freshwaters.

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figures and tables: VM, BB, GG, TC. Writing first draft: RM, BB. Writing—review and editing: SL, VB, MGaj, GB, EM, VM, GFF.

Data availability All data analyzed and described in this paper are provided in a specific repository: https://doi.org/10.6084/m9.figshare.28493057.v2.

Declarations

Conflict of interests Authors declare no conflict of interest.

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