Between darkness and light: can spring habitats provide new perspectives for modern researchers on groundwater biology? (#59853)

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- Clear, unambiguous, professional English language used throughout.
- Intro & background to show context. Literature well referenced & relevant.
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- Is the review of broad and cross-disciplinary interest and within the scope of the journal?
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- Does the Introduction adequately introduce the subject and make it clear who the audience is/what the motivation is?

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- Article content is within the Aims and Scope of the journal.
- Rigorous investigation performed to a high technical & ethical standard.
- Methods described with sufficient detail & information to replicate.
- Is the Survey Methodology consistent with a comprehensive, unbiased coverage of the subject? If not, what is missing?
- Are sources adequately cited? Quoted or paraphrased as appropriate?
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Between darkness and light: can spring habitats provide new perspectives for modern researchers on groundwater biology?

Raoul Manenti Corresp., 1, 2 , **Beatrice Piazza** 1

1 Department of Environmental Science and Policy, Università degli Studi di Milano, Milano, Lombardia, Italy

2 Parco Regionale del Monte Barro, Laboratorio di Biologia Sotterranea “Enrico Pezzoli”, Galbiate, Italy

Corresponding Author: Raoul Manenti
Email address: raoul.manenti@unimi.it

Springs are interfaces between groundwater and surface habitats and may play an important role in the study of subterranean animals. In this systematic evidence review and meta-analysis, we explore whether observations of stygobionts in springs are relevant and more common than observations of epigean animals in groundwater. We searched the Web of Science database for papers on groundwater fauna and spring fauna. For each paper we found, we recorded whether the paper reported the occurrence of typical stygobionts in springs, of surface animals in groundwater, or of the same taxa in both habitats. If so, we recorded how many such species were reported. We also recorded the scientific discipline of each study and the year of publication. Our search yielded 342 papers. A considerable number of these papers reported stygobionts in springs: 20% of papers dealing with groundwater fauna and 16% of papers dealing with spring fauna reported the occurrence of stygobionts in spring habitats. Both the number of papers that mentioned stygobionts in springs, and the number of stygobiont species that were documented in springs, were higher than equivalent measures for the occurrence of surface fauna underground. We also detected a positive relationship between year of publication and the number of reports of stygofauna in springs. To broaden the insights from biological research on underground environments, we suggest that springs should be considered not only as simple sampling points of stygobionts but also as core stygobiont habitats.

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Raoul Manenti[1,2], Beatrice Piazza[1]

[1] 1Department of Environmental Science and Policy, Università degli Studi di Milano, via Celoria, 26, 20133 Milano Italy

[2] Laboratorio di Biologia Sotterranea "Enrico Pezzoli", Parco Regionale del Monte Barro, Galbiate, Italy.

Corresponding Author:
Raoul Manenti[1]
via Celoria, 26, 20133 Milano Italy
Email address: raoul.manenti@unimi.it

Abstract

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Introduction

The zoologist Lazăr Botoşăneanu (1998) defined springs as the "doors on River Styx," the river of the Greek mythological underworld. Other biologists who study subterranean environments and groundwaters similarly consider springs to be openings that allow them to see the inhabitants of an otherwise inaccessible environment (Culver et al. 2012; Pipan et al. 2012). This view of spring habitats as windows into a different environment is particularly true in non-karst areas, where the lack of caves prevents human exploration of the subterranean realm and springs are often the only way to access groundwater organisms (Manenti & Pezzoli 2019). Springs are an interface between groundwater and surface freshwater habitats and are characterized by an interplay of both subterranean and epigean habitat features (Alfaro & Wallace 1994; Cantonati et al. 2006). However, the definition of spring is often approximate, especially in generalist texts. Although springs are widely considered to be ecotones, or soft transitions between surface and subterranean habitats, this transition may also be abrupt; indeed, the magnitude of this transition strongly depends on the morphology of the spring and can be mutable with daylight. Some springs represent an abrupt shift from the subterranean environment to the surface, whereas others, like the natural emitting caves (such as caves from which subterranean streams flow outside) of artificial draining galleries (such as galleries built to collect groundwater) , represent extended ecotonal environments (Balland 1992; White 2019). The border between the subterranean and surface environment can be particularly distinct during daytime, when it is strictly demarcated by the sun. Aside from sunlight, the differences that distinguish subterranean and surface environments in a spring, even across the few meters or centimetres that may characterize a spring with a sudden interface (Fig. 1), include the availability of trophic resources, the density of potential predators, and microclimate conditions (Barzaghi et al. 2017; MacAvoy et al. 2016; Manenti et al. 2013; Von Fumetti & Nagel 2011).

Because springs are border habitats, it can be difficult for biological studies to consider springs in their entirety; this difficulty has limited the potential for insights from springs to drive stronger advances in different fields of research. For example, studies that focus on springs often only consider a surface perspective and neglect the role played by groundwater(Manenti & Pezzoli 2019), whereas in karst areas, scientists studying the subterranean environment see springs as “access points” that can be used to sample the groundwater fauna living in different subterranean, underwater environments, such as the phreatic zone of karst aquifers (Malard et al. 2002). This latter view reflects the scarce consideration that is often given to springs and may limit a more general understanding of the ecological role of border habitats. As some studies have already suggested, transition zones are important for regulating ecosystem processes and the flow of dissolved organic material and organisms between surface (epigean) and underground (hypogean) habitats (Moseley 2009; Plenet & Gibert 1995). With this opinion paper based on a systematic review of the recent scientific literature, we aim to stimulate a change in the conception of and in the approach to springs by studies dealing with stygobionts and groundwater fauna. Particularly we want to underline that springs have the potential to reveal general patterns related to the zoology of stygobionts.

Stygobionts are obligate groundwater-dwellers; the word “stygobiont” reflects the fact that these species, and stygofauna more broadly, are “of the River Styx.” These organisms have evolved adaptations specific to the underground freshwater habitats in which they spend their entire life cycle (Trajano & De Carvalho 2017). Stygobionts often exhibit morphological features associated with their underground habitat. These characteristics, such as blindness and depigmentation, are commonly referred to as troglomorphisms (Pipan & Culver 2012; Romero 2009), and they limit stygobionts' ability to exploit surface environments. However, during night, the constraints are generally less clear and surface borders (i.e., springs) may become more permeable by stygofauna; for example, some observations of springs have reported the presence of organisms considered to be strict stygobionts (Bressi et al. 1999; Fišer 2019; Manenti & Barzaghi 2020). One such case is that of *Stygobromus* spp. amphipods, which are believed to regularly leave hypotelminorheic habitats to feed (Culver et al. 2006; Culver & Pipan 2014). Nevertheless, these findings are often viewed as exceptions or accidental events, and the use of springs is rarely mentioned as a trait of stygobiont biology. Observations of surface animals in caves have been similarly overlooked in the past (Sket 2008) and improperly seen as accidental; such "accidental" observations have recently been described for both groundwater and terrestrial subterranean habitats (Ficetola et al. 2018; Lunghi et al. 2014b; Manenti 2014).

Stygobionts are the main focus of subterranean biology and are usually studied using two distinct approaches. The first approach includes intense taxonomic investigations focused on the discovery and description of new taxa. The second approach views caves as powerful natural laboratories for evolutionary, ecological and behavioural studies on their inhabitants (Culver & Pipan 2014; Culver & Pipan 2019). The idea of caves as natural laboratories, first postulated by the speleologist Édouard-Alfred Martel (1894), has been espoused for more than one hundred years of subterranean studies (Poulson & White 1969). However, of the relatively large number of caves that were effectively used as laboratories during last century (Vandel 1964), few remain active. In addition, the outcomes of studies on stygofauna in these caves is rarely compared to insights obtained from studies of surface freshwater organisms. This is partially due to the characteristic features of stygobionts: they are often difficult to sample in deep subterranean environments and, due to their long life cycles and low fertility, they are difficult to raise in an experimental setting. Would including springs and other surface/underground border habitats in studies on subterranean biology increase the understanding of how the constraints of the hypogean environment affect the phenotypic responses and genetic makeup of stygobionts? The rationale of this paper takes origin from this question and is to suggest that a substantial inclusion of springs (and other border habitats between underground and surface) in studies on subterranean biology, can increase the understanding of principles governing exploitation and adaptation to hypogean environments.

In this paper, we investigate the perspectives of modern researchers on considering springs not only as simple sampling points, but also as core stygobiont habitats that can broaden the insights obtained from biological studies of underground environments. We specifically performed a systematic review of the recent scientific literature to understand i) the relevance of previous observations of typical stygobionts in springs; ii) if these observations vary according with study discipline and the year of publication; and iii) if these observations are more common than observations of epigean animals (i.e. aquatic surface species) in caves. By demonstrating that typical stygofauna are observed in springs more commonly than usually thought, we propose that, at least in some cases, the exploitation of border habitats be considered a non-negligible aspect of stygofauna ecology.

Survey methodology

Reviews are routinely performed in scientific studies to understand the current state of knowledge and provide future research perspectives in a given field. However, covering the whole spectrum of literature is almost impossible, especially in traditional disciplines that have a long history and a large amount of what has been improperly called “grey literature.” Without a specific and easily repeatable method, such a comprehensive review may lead to biased conclusions.

To avoid bias, many scientific fields have largely started to favour the use of systematic evidence reviews (Acreman et al. 2020). We therefore performed a systematic review to find focused data that addressed our three aims (Table 1). For this review, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page & Moher 2017), and we searched the Web of Science database for peer-reviewed papers on both stygofauna and fauna living in spring habitats. The Web of Science database contains metadata for high-impact scientific articles published since 1965. We used two search strings designed to find all articles in the database that might contain observations of fauna in both caves and springs. Our search was conducted in May 2020 from Milano, Italy, using the keywords “groundwater fauna” (GF) and “spring fauna freshwater” (SFF) and searching them by topics.

We initially screened the articles that met our search criteria by discarding articles that were not clearly related to our study aims. We rejected articles about botany, palaeontology, geology, and all their associated subdisciplines (paleoecology, stratigraphy, geomorphology, etc.), as well as articles about subterranean environments or groundwater that did not mention animals. The articles we found using the key words “spring fauna freshwater” were more difficult to screen; for the most part, the authors of these articles did not specify if their study species were part of stygofauna or not. We therefore discarded these articles only if they were not related to our study aims (e.g., papers about estuaries, palaeontology, or related topics) or if the authors provided clear evidence that the study species were not cave-dwellers.

We additionally discarded several articles that dealt strictly with agricultural sciences, biogeochemical cycles, the impacts of various pollutants (crude oils, perchlorate, etc.) on groundwater, or other environments strongly connected to groundwater (i.e., all surface water environments), but did not mention the finding of stygofauna or epigean fauna. Articles that addressed single species or taxa that are not stygofauna or typical spring fauna and have no hypogean representatives (e.g., (Rechulicz 2011) treated *Pseudorasbora parva* and (Vilenica et al. 2016) treated mayflies), and articles concerning terrestrial environments, estuaries, swamps, mangroves, streams, rivers, lakes, and all saltwater environments, were similarly discarded. (See Table 1 for more detailed information on the article selection procedure). After this first screening, we performed a second selection procedure in which we removed any articles that were unavailable or were written in a language other than English.

From the papers we collected the information listed in Table 1, including the typology of the study, distinguishing between ecology, taxonomy behaviour, conservation and fauna assessment and considering that the same paper could belong to multiple categories.

Statistical analyses

To assess the relationships between features of the selected documents and the occurrence of stygofauna in springs, we built a series of generalized linear models (GLMs) with binomial error distributions.

First, we assessed if the fact that a paper reported the occurrence of stygobionts in springs, the occurrence of surface fauna in groundwaters, or the contemporary occurrence of a stygobiont in both groundwaters and springs, was related to the paper's field of study. Reported occurrences were used as the dependent variable, and the study disciplines (ecology, taxonomy, faunal assessment, and conservation) were used as fixed factors. We similarly built GLMs with the same dependent variable but with publication year and the search term as independent variables.

Second, we built GLMs to test two distinct hypotheses: (1) that the number of mentions of stygobiont species in springs is higher than the number of mentions of surface species in groundwater; and (2) that missing information is different among the two situations. Both hypotheses were tested only for papers selected with the keyword "groundwater fauna" to avoid biases associated with the fact that studies found using the keyword "spring fauna freshwater" may not have sampled underground habitats. For the first test, we used the number of species mentioned by each paper as a dependent variable, including both stygofauna found in springs and surface fauna found underground. The type of observation (stygofauna in springs vs. surface fauna in groundwater) was used as a fixed factor. For the second test, we defined the dependent variable as whether it was possible to assess the number of species mentioned in a study, including both types of observations (stygofauna in springs and surface fauna in groundwater). The type of observation was used as a fixed factor, as before. To avoid overdispersion bias, we built both models using a type 2 negative binomial error distribution in the package glmmTMB (Brooks et al. 2017).

We used a likelihood ratio test to assess the significance of all the fixed factors included in each GLM (Bolker et al. 2008). All analyses were performed in the R 3.6.3 environment.

Results

We retrieved 824 potentially relevant papers after removing duplicate articles. After removing articles based on the first selection criteria described above, there were 415 potentially relevant documents. After the second selection procedure, we obtained 342 papers: 275 derived from the search term "groundwater fauna" (GF) and 67 from the search term "spring fauna freshwater" (SFF). Many papers found using the "groundwater fauna" search did not specify the sampling site for the taxa considered, and many papers found using the "spring fauna freshwater" search did not clearly identify if they sampled stygofauna or not. Overall, 57 papers (representing 19% of the papers with information on sampling habitat) reported the occurrence of stygofauna in springs, 37 (11.7%) reported the occurrence of typical surface fauna underground, and 33 (11%) reported the same taxa in both springs and groundwater (Table 2). With respect to our search terms, 20% of papers dealing with GF and 16% of papers dealing with SFF described the occurrence of stygobionts in spring habitats.

There were 45,375 species mentioned across all papers we retrieved. Of these, 138 were stygobionts observed/sampled in springs and 46 were surface species observed in subterranean habitats.

The study disciplines covered by the papers were mainly ecology (196 papers) and faunal assessments (177 papers). Four papers were behavioural studies, and 24 papers addressed conservation concerns. There were 194 papers that encompassed multiple fields of study.

Our first analysis revealed that faunal assessments are significantly more likely to report the occurrence of surface fauna in groundwater, whereas taxonomic studies are more likely to report the occurrence of the same taxon in both environments (Table 3). We did not detect any relationship between the discipline of a paper (ecology, taxonomy, etc.) and the reported occurrence of stygofauna in springs. However, we did detect a positive relationship between the year of publication and the reports of stygofauna in springs (χ^2=4.53, P=0.03). Papers selected using the SFF search term were significantly less likely to report the occurrence of surface taxa in groundwaters (χ^2=4.09, P=0.04).

GLMs performed on papers selected using the GF search term revealed that the number of mentions of stygobiont species in springs is higher than the number of mentions of surface fauna underground (χ^2=4.19, P=0.04). However, there is also less information available on whether stygofauna have been observed in springs compared to whether surface species have been recorded in groundwaters (χ^2= 14.08, P<0.001).

Discussion and perspectives

We retrieved 824 potentially relevant papers after removing duplicate articles. After removing articles based on the first selection criteria described above, there were 416 potentially relevant documents. After the second selection procedure, we obtained 342 papers: 275 derived from the search term “groundwater fauna” (GF) and 67 from the search term “spring fauna freshwater” (SFF). Many papers found using the “groundwater fauna” search did not specify the sampling site for the taxa considered, and many papers found using the “spring fauna freshwater” search did not clearly identify if they sampled stygofauna or not. Overall, 57 papers (representing 19% of the papers with information on sampling habitat) reported the occurrence of stygofauna in springs, 37 (11.7%) reported the occurrence of typical surface fauna underground, and 33 (11%) reported the same taxa in both springs and groundwater (Table 2). With respect to our search terms, 20% of papers dealing with GF and 16% of papers dealing with SFF described the occurrence of stygobionts in spring habitats.

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Discussion and perspectives

Our systematic review revealed that there are more papers about stygofauna available on Web of Science than there are papers addressing fauna and springs. Because the word “spring” is a homograph with multiple meanings, our initial search retrieved many papers that were ultimately discarded because they did not discuss fauna and spring habitats. Preliminary literature searches performed using synonyms of “spring” and/or terms that define specific spring habitats, such as “sources” or “seepage,” resulted in fewer papers. Most of these papers were already included in our analysis; however, the few that were not could be used in future study with a larger set of papers. Someone could disagree as it is likely that our research missed some papers and that further keywords should have been added, for example: stygob*, ecoton*, hypogean, subterranean etc, but they would have increased the number of papers dealing with stygofauna without significant increase in the number of papers related to spring fauna. The large difference in the number of papers obtained with the two search terms, GF and SFF, underscores the fact that the fauna of spring habitats have received much less attention not only than the inhabitants of lakes and streams/rivers, as already pointed out by previous studies (Cantonati et al. 2011), but also than stygofauna.

We limited our review to articles archived in Web of Science; this approach was more conservative because it included only relatively recent papers published in indexed, high-impact journals that perform selective peer review. An analogous review could be performed using the Google Scholar database or a more exhaustive search of grey literature in online and physical repositories. It is possible that the older, descriptive papers archived in these databases may have reported stygofauna in springs, but it is also possible that some form of bias could arise from using older literature that has not been rigorously peer reviewed. The effects of database selection should therefore be investigated in the future.

Using both GF and SFF as search terms, we found papers that mentioned the occurrence of stygofauna in springs, of typical surface fauna in groundwaters, and of the same taxa in both environments. The number of papers that reported stygofauna in springs, as well as the number of stygobiont species that were documented in springs, represented only a fraction of the total papers and documented species but were nevertheless non-negligible. This pattern was not linked to any specific field of study; though taxonomic studies were non-significantly more likely to report stygofauna in springs.

Springs have been recognized as relevant habitats for studying stygobionts since the beginning of the 19^{th} century. Most of the major subterranean biologists devoted at least some of their studies to spring habitats (Culver et al. 2012; Culver et al. 2014; Vandel 1920), and Albert Vandel, the founder of one of the most popular subterranean laboratories in the world (Botosaneanu 1980), stated in 1920 that a systematic study of spring habitats could furnish important insights for solving some of the evolutionary questions posed by cave-dwelling animals (Vandel 1920). However, this concept appears only in Vandel's conclusions and is not further developed; the idea that springs are just sampling points in non-karst areas largely prevails throughout the rest of the paper (Vandel 1920).

Stygobionts are known to colonize the mixed assemblages of organisms residing in springs via emigration from groundwaters (Malard et al. 2009; Malard et al. 2002). Typical stygobionts may be more or less permanently detected outside the spring outlet, where they can exploit different microhabitats (Malard et al. 2002; Mathieu et al. 1994). This is especially true when there is a stable supply of immigrants from karst groundwater (Mathieu et al. 1999). Our results revealed that typical stygofauna have been reported in springs more frequently in recent years; this means that, with respect to older studies, researchers are paying more attention when reporting data on sample collection habitats, regardless of their study discipline (ecology, taxonomy, conservation, or faunal assessment). However, in the papers that we collected, the occurrence of stygobionts in springs was often reported as either an effect of the sampling method or an occasional finding. None of the papers assessed patterns in the use of springs by stygobionts. This is true also for some papers that we missed with our search but that are well known in spring literature. As an example (Rouch 1986) defined "the hemorrhage" the flow of stygofauna pushed out from aquifers during high discharge periods through springs, erroneously considering this only as a passive mechanism. In more recent times, some papers were devoted to spring discharge and the passive presence of stygobionts being flushed from "conductive" or "capacitive" aquifers has been (Di Lorenzo et al. 2005); other large-scale ecological surveys of springs demonstrated that in mountain areas, where species richness of stygobionts is usually poor due to the effect of Quaternary Galciations, their occurrence seems low or occasional in springs (Stoch et al. 2011), suggesting that the geographical location of springs matter and could be considered in future systematic reviews dealing with springs. Springs are also being studied with recent and 'modern' approaches like DNA metabarcoding techniques and eDNA that can allow to detect the presence of stygobionts in springs (Niemiller et al. 2018; Thomsen et al. 2012) and be used in the future to assess the patterns that determine this occurrence.

The occurrence of a stygobiont species, or a species that is strictly linked to a hypogean groundwater habitat for its life cycle, in an epigean spring habitat, underlines a contradiction that might reflect the human conceptual limit of understanding borders. The human perception of limits and boundaries may be biased, as humans may recognize or emphasize abrupt distinctions when they do not exist (Pirni 2016; Sturz & Bodily 2016). Our results demonstrate that, at least for some stygobionts, border habitats and adjacent areas are an important part of the range and biology of stygofauna, and a proper consideration of these habitats in subterranean biology studies could provide larger perspectives. For example, stygobiont populations or individuals that exploit springs more or less permanently are exposed to different constraints and advantages than populations or individuals that exploit deeper aquifers. Selective pressures may therefore act differently, at least for the individuals living in springs or at the interface between subterranean and epigean habitats. For example, different species and/or populations of the genus *Niphargus*, which shows typical features of stygobionts including depigmentation and the absence of eyes, have the unique ability to detect light (Fišer et al. 2016). This ability has been associated with the need to distinguish the border between surface and subterranean environments and avoid risky surface habitats (Fišer et al. 2016) where UV rays may be dangerous for a depigmented animal. However, surface habitats may also be advantageous by furnishing higher trophic resources and, at night, they are not exposed to UV light. Several studies have reported *Niphargus* amphipods in border habitats (Fiser et al. 2007; Manenti & Pezzoli 2019; Marković et al. 2018). Is light perception the same between individuals from borders and individuals from deeper aquifers? Are there evolutionary adaptations for exploiting not only deep subterranean habitats but also border habitats at the interface with the surface? These questions are applicable to all stygobionts that are recurrently found in springs. Considering border habitats in addition to deeper subterranean environments therefore has the potential to double the insights obtained from studies of stygobionts. These insights could be used not only to disentangle evolution from the adaptations to the selective pressures of groundwater habitats but also to characterize the physiological responses stimulated by the interaction with different environmental conditions.

Our results further demonstrate that stygofauna are reported in springs more frequently than surface fauna are reported in groundwater, in terms of both number of papers and overall numbers of species. In recent years, a growing body of literature has shown that even the occurrence of surface species in caves is often not accidental (Lunghi et al. 2014a; Lunghi et al. 2017), a finding that has important implications for the communities of shallow subterranean habitats (Kozel et al. 2019; Lunghi 2018; Lunghi et al. 2020; Salvidio et al. 2020; Silva et al. 2020). If stygofauna occur in springs and adjacent microhabitats more commonly than surface fauna occur underground, it is likely that, at least for some stygobionts, the use of the surface environment is not accidental.

Further systematic reviews and analyses of the literature on spring fauna could be performed to investigate the countries where the largest number of studies on springs were carried out, the most studied taxa and the most studied functional traits. Moreover, marine caves can further support the idea that springs are just an ecotone that should also be studied from an ecological viewpoint (Romero, *in litteris*). For example, there are sea fish species that enter and exit marine caves playing a significant role in those environments' ecology. That is the case with the cardinal fish *Apogon imberbis*. This is a small-sized fish distributed along the eastern Atlantic coast from Morocco to the Gulf of Guinea, including the Azores. It can be found as solitary or forming schools and is common in small crevices to marine caves, where they can be found in large densities. They show no troglomorphisms, yet they play a significant role in transferring organic material to these marine caves as mysid crustaceans do (Romero, *in litteris*). Like bats, they tend to stay in the shelters during the day and leave the caves at night, presumably for feeding (Bussotti et al. 2003).

The occurrence of stygobionts in springs could affect both the dynamics of boundary habitats and, at the level of the whole stygobiont population, the intrinsic traits of the species. There are several different perspectives for how a stronger conceptual inclusion of springs in subterranean research may provide additional insights on subterranean biology. First, springs may favour intraspecific variation that could be assessed by comparative experimental studies, which would benefit studies of intraspecific dynamics between boundaries and deep areas. Second, springs can inform studies of the processes that promote adaptation to and colonization of border habitats, as research on springs could be used to distinguish possible phenotypic plasticity from local adaptations. Third, given the view of springs as useful laboratories, devoting space and infrastructure at the entrance to subterranean environments could provide important experimental opportunities.

Conclusions

Even if the transitional and ecotonal role of springs is known and studied since several decades, and the term GDE (Groundwater Dependent Ecosystems) applied to springs allows to study the connected network of surface and subterranean ecosystems following the 'holistic' approach suggested by (Linke et al. 2019), these concepts are rarely translated in ecological and evolutionary studies dealing with groundwater animals. The results of our systematic review broadly suggest that springs and other boundaries with surface environments should be considered and investigated as part of subterranean habitats and of the biology of at least some stygobionts. Studies of groundwater environments and stygobiont biology that do not consider springs may furnish only a limited perspective on subterranean environments. The study of groundwater-adapted organisms in subterranean aquifers has the potential to reveal new insights in several scientific fields (Pipan & Culver 2013; Reboleira et al. 2011), but the study of the boundaries of groundwater environments, such as springs, is not only equally important, but even necessary to understand the zoology ecology and evolution of groundwater fauna.

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Table 1(on next page)

Table 1

Table 1. Search terms and inclusion/exclusion criteria used to describe published evidence of stygobionts in springs and to answer specific questions.

Table 1

Categories	Restrictions applied
Number of species mentioned	If clearly stated for all taxa considered in the study
Stygofauna found in springs	If clearly stated that the species found in springs are stygobites
Number of stygofaunal species in springs	If the number of stygobite species found in springs is clearly stated for all taxa considered in the study
Surface fauna found underground	If clearly stated that the species found underground are of epigean origin
Number of surface species found underground	If the number of epigean species found underground is clearly s tated for all taxa considered in the study
Species found both in caves and springs	If clearly stated that the species found both in caves and springs are epigean or stygobites
Number of species in both (caves and springs)	If the number of stygobites or epigean species found in both is clearly stated for all taxa
Ecology	Yes/no, depending on whether the paper provides original ecological information (habitat of occurrence, environmental drivers etc..)
Taxonomy	Yes/no, depending on whether the paper provides original taxonomic data
Behavior	Yes/no, depending on whether the paper tests/reports original behavioral information/observations
Conservation	Yes/no, depending on whether the paper explores original conservation/restoration problems or actions
Faunal assessment	Yes/no, depending on whether the paper is mainly devoted to assess faunal composition of spring/groundwater habitat

Table 1. Search terms and inclusion/exclusion criteria used to describe published evidence of stygobionts in springs and to answer specific questions.

Table 2(on next page)

Table 2

Table 2. Number of papers reporting observations of stygofauna in springs, of surface fauna in groundwaters, and of the same taxa in both environments. Papers are divided based on the key words used for the systematic review: GF, groundwater fauna; SFF, spring freshwater fauna.

Table 2

		Total	GF	SFF
Stygofauna in springs	YES	57	49	8
	NO	235	195	40
	Information missing	50	31	19
Surface fauna underground	YES	37	34	3
	NO	278	220	58
	Information missing	27	21	6
Same taxa both in springs and groundwaters	YES	33	30	3
	NO	266	214	52
	Information missing	43	31	12

Table 2. Number of papers reporting observations of stygofauna in springs, of surface fauna in groundwaters, and of the same taxa in both environments. Papers are divided based on the key words used for the systematic review: GF, groundwater fauna; SFF, spring freshwater fauna.

Table 3(on next page)

Table 3

Table 3. The reported occurrence of stygofauna in springs, of surface fauna in groundwaters, and of the same taxa in both habitats shown as a function of study discipline. Relationships were assessed using generalized linear models (GLMs) followed by a likelihood ratio test. Significant relationships are reported in bold.

Table 3

	Research discipline	Estimate	SE	LRT	P
Stygofauna in springs	Ecology	0.27	0.34	0.64	0.42
	Taxonomy	0.75	0.39	3.67	0.06
	Faunal assessment	0.24	0.35	0.48	0.49
	Conservation	0.67	0.53	1.50	0.22
	Behavior	-13.84	834.76	0.98	0.32
Surface fauna underground	Ecology	0.02	0.41	0.00	0.96
	Taxonomy	0.27	0.50	0.29	0.59
	Faunal assessment	1.09	0.45	6.62	**0.01**
	Conservation	0.51	0.59	0.68	0.41
	Behavior	1.30	1.20	0.94	0.33
Same taxa both in springs and groundwaters	Ecology	0.09	0.43	0.04	0.84
	Taxonomy	0.91	0.45	3.96	**0.04**
	Faunal assessment	0.06	0.44	0.02	0.89
	Conservation	0.77	0.60	1.45	0.23
	Behavior	-13.15	839.90	0.51	0.48

Table 3. The reported occurrence of stygofauna in springs, of surface fauna in groundwaters, and of the same taxa in both habitats shown as a function of study discipline. Relationships were assessed using generalized linear models (GLMs) followed by a likelihood ratio test. Significant relationships are reported in bold.

Figure 1

Figure 1

Diagram of a spring showing differences between surface and groundwater habitats during day (A) and night (B). White silhouettes represent stygobionts, black silhouettes represent potential predators (fish and salamanders), and brown silhouettes surface aquatic invertebrates. Drawing is modified from Andrea Melotto and Benedetta Barzaghi (unpublished).

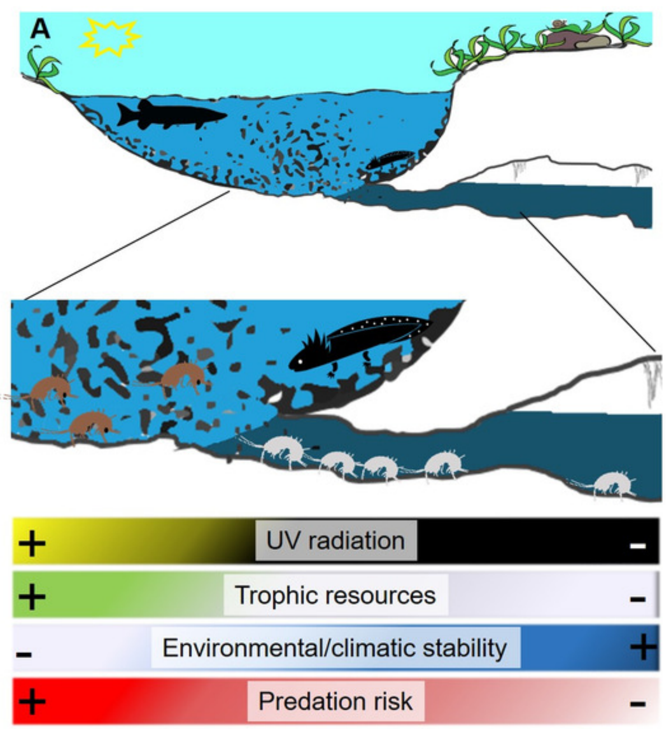
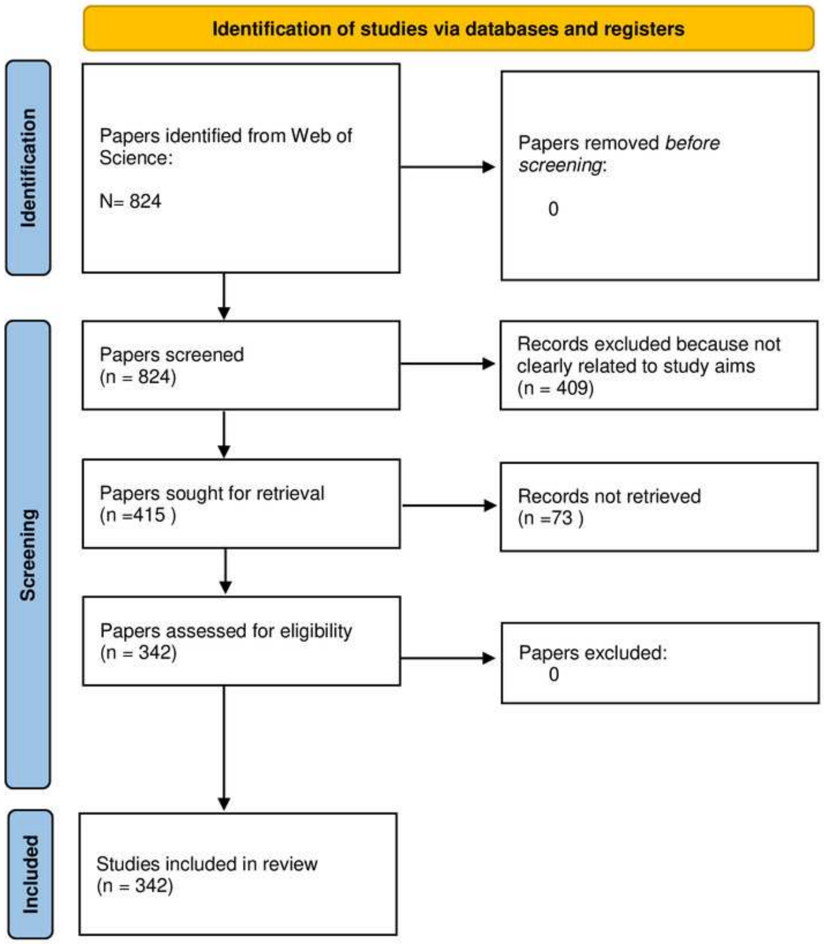


Figure 2

Figure 2

Figure 2 PRISMA 2020 flow diagram for the systematic reviews which included search of Web of Science database only.


Identification of studies via databases and registers
Identification
Papers identified from Web of Science:
N= 824
Papers removed *before screening*:
0
Screening
Papers screened
(n = 824)
Records excluded because not clearly related to study aims
(n = 409)
Papers sought for retrieval
(n =415)
Records not retrieved
(n =73)
Papers assessed for eligibility
(n = 342)
Papers excluded:
0
Included
Studies included in review
(n = 342)