[Running head left:] Raoul Manenti & Benedetta Barzaghi

[Running head right:] Diel activity of amphipods in springs

Diel activity of *Niphargus* amphipods in spring habitats

BY

Raoul Manenti[1,2,3]) and Benedetta Barzaghi[1,2])

[1]) Department of Environmental Science and Policy, Univeristy of Milan, Via Celoria, 26, I-20133 Milan, Italy

[2]) Laboratory of Subterranean Biology "Enrico Pezzoli", Regional Park of Mount Barro, I-23851 Galbiate, Italy

ABSTRACT

Among crustaceans, numerous aquatic species are obligate groundwater-dwellers, i.e., stygobionts; their most common adaptations are the absence of eyes and a general depigmentation. Among the most widespread Eurasian stygobionts are the amphipods of the genus *Niphargus*. They are reported not only from groundwaters but also from groundwater-fed springs, where the abundance of food is higher, but where they also experience the constraint of UV radiation during the day. The aim of this study was to assess if in spring habitats *Niphargus* amphipods show diel activity, in particular if they are able to exploit the resources during the night.

During two consecutive years, we evaluated, with both day and night surveys, the

---

[3]) Corresponding author; e-mail: raoul.manenti@unimi.it

abundance of *Niphargus* sp. individuals at four springs in NW-Italy. We performed surveys both visually and with dip-nets and we evaluated the relationship between *Niphargus* sp. abundance and the number of potential predators.

We detected a significant difference between day and night in the abundance of *Niphargus* sp.: during the night the number of individuals was substantially higher. No significant relationship was observed between *Niphargus* sp. abundance and the abundance of potential invertebrate predators.

The broad implication of this study is that the number of active detectable *Niphargus* in springs is higher at night than during daytime, regardless of the number of potential predator species occurring. This suggests that one of the major constraints for the exploitation of spring habitats by *Niphargus* amphipods is the UV radiation, and that specific adaptations favouring diel activity in border habitats, like springs, may have evolved in these basically stygobiont species.

Key-words. — Groundwater, springs, seepage, *Stygobromus*, *Niphargus*, dispersal, Amphipoda, "fontanile", spout, fountain, stygobite

RIASSUNTO

Tra i crostacei numerose specie acquatiche dipendono dalle acque sotterranee per il completamento del ciclo vitale, ovvero sono stigobie. Tali specie mostrano spesso adattamenti morfologici e fisiologici quali la mancanza di pigmentazione e l’assenza di occhi. Tra gli stigobi maggiormente diffusi in Eurasia vi sono gli anfipodi del genere *Niphargus*; essi si rinvengono non solo nelle acque sotterranee propriamente dette, ma anche negli ambienti sorgivi dove le risorse trofiche sono relativamente maggiori, ma dove si trovano ad esperire gli effetti delle radiazioni UV durante il giorno.

Lo scopo di questo lavoro è stato di verificare se gli anfipodi del genere *Niphargus* osservati in ambiente sorgivo mostrino variazioni nell’attività giornaliera con un’abbondanza maggiore di individui attivi durante la notte.

Nel corso di due anni consecutivi abbiamo conteggiato sia di notte, sia di giorno l’abbondanza di *Niphargus* sp. in quattro sorgenti del Nord-Italia. Abbiamo effettuato campionamenti visuali e tramite retino e valutato anche l’effetto dell’abbondanza di potenziali predatori.

I risultati ottenuti mostrano che le abbondanze di specie di *Niphargus* attivi siano significativamente maggiori di notte; al tempo stesso non vi è relazione significativa con il numero di potenziali predatori presenti.

Questa ricerca fornisce importanti indicazioni del fatto che in ecotoni acqua superficiale/acqua sotterranea, come nel caso delle sorgenti, le radiazioni UV possano rappresentare uno dei principali fattori che limitano la dispersione dei crostacei stigobi; sottolinea inoltre come, adattamenti quali la capacità di percepire gli stimoli luminosi, potrebbero essere insorti per favorire la colonizzazione di ambienti acquatici superficiali alimentati direttamente dagli acquiferi.

INTRODUCTION

In freshwater habitats there is a high diversity of crustaceans that in part mirrors the miscellaneous array of micro-habitat typologies that are available to colonization (Gilbert et al., 2015,; Dodds et al., 2019). In freshwater ecosystems, particularly crustaceans and other invertebrates perform various ecological functions of fundamental importance (Collier et al.; 2016, Manenti et al., 2019; Cantonati et al., 2020). Aquatic invertebrates are also regarded as good indicators of the general status and of the level of pollution of freshwater habitats. For these reasons, several studies have been conducted to assess the features of the invertebrate communities, and several indexes, either based on the number, on the sensitivity, or on the function of the invertebrate taxa, have been developed for freshwater habitats (Koperski, 2011). The assessment of macroinvertebrate assemblages in fresh waters strongly relies on the sampling techniques applied (Florencio et al., 2012). Moreover, several studies have shown that for some taxa, the number of individuals sampled may vary between night- and daytime (Rincon & Lobon Cervia, 1997; Cereghino & Lavandier, 1998; Florencio et al., 2012). However, all methods used to sample freshwater benthic invertebrates for assessing biological indexes, are applied during the day (Cao et al., 1997; Smith et al., 2007; Leunda et al., 2009; Musonge et al., 2019) and differences determined by macrobenthos diel activity are scarcely considered in most assessments of the representative species of macroinvertebrate communities.

Several factors may determine differences in macroinvertebrate diel activity; in general, all the aspects under the constraints of Darwinian natural selection as: food availability, predation risk and other inter- and intra-specific interactions may concur to determine differences in the density of freshwater crustacean species across day- and night-time (Kusano & Kusano, 1991; Elliott, 2002; Manenti & Barzaghi, 2020). Differences in the diel activity of macroinvertebrates, including freshwater crustaceans, can be particularly important, and therefore interesting to be assessed, in spring habitats. Springs are interfaces between groundwaters and surface fresh waters, with both the subterranean and the epigean habitat features that interplay in characterizing each spring (Alfaro & Wallace, 1994; Cantonati et al., 2006). Surface fresh waters and groundwaters differ mainly for the abundance of trophic resources and of potential predators for detritus-feeder species, which are slightly higher at the surface, as well as for the stability of the water flow and microclimatic conditions that are generally higher underground (Von Fumetti & Nagel, 2011; Manenti et al., 2013b; Culver & Pipan, 2014; Barzaghi et al., 2017).

The community of macroinvertebrates occurring in springs can be composed of both typical epigean species for which springs are the upstream distribution limit, and typical groundwater-dwelling species (i.e., stygobionts) for which springs are the downstream distribution limit (Cantonati et al., 2011; Manenti & Pezzoli, 2019). Together with these species, also taxa more exclusively linked to springs (so-called crenobionts) can coexist (Di Sabatino et al., 2003; Pezzoli, 2010). Normally, stygobionts live in groundwater habitats that are essential for completing their life cycles (Culver & Pipan, 2014); however, stygobiont species may also enter spring habitats in different ways, depending on the features of the species or species-groups (Vandel, 1920, 1964; Niemiller et al., 2008; Manenti & Pezzoli, 2019). The most common way of occurrence in springs is through drift with the water flow that may flush stygobionts out of their primary habitat and make them entering another one, i.e., a spring habitat (Mathieu et al., 1994; Mathieu et al., 1999; Malard et al., 2002; Bottazzi et al., 2008). The drift of stygobionts, although strongly related to features and changes of the water flow (Kureck, 1967) is connected with the species' dispersal ability and must not be regarded only as a passive or purely occasional mechanism (Mora et al., 2013; Mori et al., 2015; MacAvoy et al., 2016). Other than drift, also active movements of stygobionts from the groundwater to springs have been documented (Bressi et al., 1999; Niemiller et al., 2010), while other factors, not necessarily related to water flow changes, may explain why and when stygobionts are more or less abundant at the site where the spring emerges.

Borders between groundwater and surface waters can be particularly definite, especially during daytime when sunlight strictly demarks the limits (fig. 1). Due to UV radiation, epigean habitats can be considered harsh environments that pose important constraints, especially to stygobionts that are usually deprived of pigments (Culver et al., 2012; Culver & Pipan, 2019). During the night, spring habitats should become in theory more attractive for stygobionts due to the higher possibility of finding food in comparison to groundwaters. However, also the predation risk for stygobionts can be higher, as surface mesopredators can be more active during night-time (Huhta et al., 2000; Manenti et al., 2015). Despite the important role that night-time exploitation of springs by stygobionts may play, it is difficult to find papers investigating variation of stygobiont diel activity at the interface between groundwaters and surface fresh waters, and most of these have been published in the 19th century and their results are currently confined to general statements in the so-called "grey literature".

Crustaceans are important representatives of the groundwater fauna (Romero, 2009); among the most widespread stygobionts are the amphipods of the genus *Niphargus* Schiødte, 1849. *Niphargus* is the most diversified genus of freshwater amphipods, with more than 420 described species (Danielopol et al., 1999; Robertson et al., 2009; Horton et al., 2019). *Niphargus* species primarily inhabit groundwaters, even if some mainly epigean populations and species are known (Copilas-Ciocianu et al., 2017; Hudec et al., 2017; Marković et al., 2018). They have also frequently been reported for spring habitats (Fišer et al., 2007; Marković et al., 2018; Manenti & Pezzoli, 2019) where they find a higher availability of trophic resources than in groundwaters (Kureck, 1967; Dhomps-Avenas & Mathieu, 1983). *Niphargus* amphipods show typical features of stygobionts, such as absence of eyes and depigmentation, but at least some species have the capability to detect light and show negative phototaxis (Blume et al., 1962; Borowsky, 2011; Fišer et al., 2016). This capability has been associated with the necessity of distinguishing and avoiding risky surface habitats (Fišer et al., 2016).

In the past century, some studies have been performed on the variation of the number of *Niphargus* amphipods that had drifted into springs from groundwaters during the day and night (Müller et al., 1963; Kureck, 1967). They show that the number of updrifted individuals is higher during the night: Kureck (1967) in a study of 14 springs around the city of Schlitz (Germany) reported a maximum total number of 866 individuals of *N. aquilex* Schiödte, 1855 drifted during the night over a period of 11 days at a spring, while during daytime he recorded a maximum total number of 312 individuals at the same site for the same period; Müller et al. (1963) show a graph reporting numbers of drifted *N. aquilex* individuals, collected both during day and night in the same spring for nine days: the average (±SE) of drifted *N. aquilex* is 12.6 ± 0.9 individuals during the night, versus 2.1 ± 0.8 individuals during the day. Moreover, these studies show that during the day updrifted individuals actively migrate back to the groundwater (Kureck, 1967).

However, assessing spring exploitation by freshwater invertebrates on the basis of drift samplings may be challenging, because drift itself can be considered both a passive mechanism affected by water flow changes and also an antipredator response. Early studies on *Niphargus* evidenced that they have only scarce ability to withstand the water flow (Ginet, 1960), while Kureck himself showed that at least part of the changes in the number of individuals that he collected in some springs was linked to changes in water flow (Kureck, 1967). Moreover, in first order streams inhabited by fire salamander larvae (*Salamandra salamandra* (Linnaeus, 1758)), the drift of invertebrates (and particularly amphipods) is higher during the night, not necessarily because all these invertebrates are more active during the night, but certainly because the salamanders are (Oberrisser & Waringer, 2011).

Assuring differences in the diel activity of *Niphargus* in spring habitats could provide new insights in the fact that the ability of light detection could be associated to the possibility of exploiting surface resources during the night, when UV rays are not a constraint. But more specifically, the primary question is contained in the possibility that adaptations to exploit habitats at the interface with the surface have evolved in stygobionts, which for certain can not remain as as an only casual or weak interest for zoologists. This study, therefore, is nothing less than a first attempt to assess if *Niphargus* amphipods show diel activity and compare the role of different conditions in affecting the abundance that can be detected in spring habitats. In particular, we hypothesize that if their major constraint for the exploitation of the trophic resources occurring in surface habitats is related to UV radiation, as their capability to detect light seems to suggest, during the night the number of active, detectable *Niphargus* in springs would be higher, irrespective of the number of potential predator species or individuals present.

MATERIAL AND METHODS

Study system

Several typologies of spring ecosystems may exist, from stable ecotones to temporary habitats with abrupt interfaces between underground and surface (Manenti & Pezzoli, 2019; White, 2019). For this study we selected permanent spring habitats in which the water flow is generally constant, such as the so called “fontanili” springs; these are common habitats in the lowlands of the Po River Plain (N Italy). Fontanili are a kind of quasi-natural springs fed by groundwater flow through sudden interfaces that were managed by humans for numerous centuries (Balderacchi et al., 2016). In particular humans pushed pipes in the substrate to facilitate groundwater outflow; pipes work like fountain spouts and drive the main outflow of groundwater, but usually also not-managed water emergences occur. The area in which pipes and the other water emergences occur is characterized by a more or less large lentic water body, called “head” of the “fontanile”. In this waterbody, rich organic sediment and a cover of abundant aquatic macrophytes can occur. Generally, fontanili springs persist in a specific range of the Po River Plain (fig. 2), where consistent deposits of different origins, from alluvial quaternary to marine pliocenic, exist (De Luca et al., 2020). Fontanili are fed by the shallow, unconfined aquifer that is hosted in the alluvial deposits (De Luca et al., 2020) and that usually reaches the surface in that particular range. However, occasionally, similar emergences of the water table can occur further north. In Lombardy, different species of *Niphargus* have been observed; all of them are stygobionts or interstitial, except *Niphargus elegans* Garbini, 1894, which has been detected in surface waters only inside the fontanili range (Stoch, 2000).

Here, we studied four “fontanili” springs in Lombardy that occur far north of the fontanili range and that are close to the city of Erba in the Como district along the River Lambro (fig. 2).

These four springs occur on alluvial sediments in the piedmont of a relatively large karst area inhabited by the species *Niphargus ambulator* Karaman, 1975, a mainly scraper-feeder, and *N. tridentinus* Stoch, 1998, a primarily predatory species (Karaman, 1975; Stoch, 2000). At the four study sites, the occurrence of populations of *Niphargus* sp. is known since some years (Manenti et al., unpubl.); their general morphology does not differ dramatically from that of *N. ambulator*, however their taxonomic status has not been verified yet and further investigations by taxonomists will be necessary to properly identify these populations. A diurnal sampling, performed by E. Pezzoli on 24 March 2019 in one of the sites using Bou-Rouch pumping (site “Erba2”, fig. 2) revealed *Niphargus* sp. occurrence in the groundwater that was feeding the spring. The four fontanili heads that we studied show a similar content of organic sediment and macrophyte cover; in all of them groundwater flows not only from the pipes but also from other small, not managed emergences.

At the same four spring sites we recently assessed the role of the landscape in affecting the activity of some epigean freshwater invertebrates (Manenti & Barzaghi, 2020) without considering stygobiont taxa.

Surveys

During the winter months, from December 2017 to February 2018, and from January 2019 to February 2019, we performed in each site repeated surveys both by day and during the night (supplementary table I) along straight transects. The transects were all 1 m wide, but varied in length and water depth depending on the specific features of the site (average ± SE length = 4.3 ± 0.7 m; average ± SE water depth = 48.7± 12.2 cm). We considered daytime surveys those performed from 10 am to 5 pm, and night-time surveys those performed from 8 pm to 1 am. We performed the surveys always along the same transects at the spring's head. We divided two springs into two transects (so we sampled six transects in total). We selected transects at a minimum distance of 60 cm from the pipes' spouts. To perform surveys, we avoided the use of drift nets, that are often employed to sample stygobiont animals in spring habitats (Malard et al., 2002), because it would have prevented to understand if individuals of *Niphargus* sp. were just passively drifted by groundwater flow, or were actively moving in the spring habitat.

Each survey was divided into two phases. We first assessed visually the occurrence and the number of *Niphargus* sp. individuals, by carefully checking, for 20 minutes, all the elements of the substrate. In order to facilitate identification and to distinguish eventual macroscopically different specimens, we employed an Olympus TG-4 waterproof camera with macro enlargement. After those 20 minutes we additionally performed a dip-net sampling for macroinvertebrates. Net samplings lasted 10 minutes in each transect and were performed by intense movement of the substrate. All invertebrates collected, including *Niphargus* individuals, were released in the transect of origin after having been counted and recognized at species, genus, or family level according to the guidelines for the Italian Biotic Index assessment (Ghetti, 1997).

Statistical analyses

We used random-effect generalized mixed models (GLMMs) to assess the relationships between the relative abundance of *Niphargus* sp. and the environmental features (Barker et al., 2017). Generalized mixed models yield reliable estimates of the relationships between the relative abundance of species with imperfect detection and environmental conditions (Barker et al., 2017). We used a negative binomial distribution to account for overdispersion as we had different 0 occurrences. As dependent variable, we considered the number of *Niphargus* sp. individuals observed for each transect at each survey. We included the moment of observation (day/night), the sampling method (visual/net) and the log-transformed number of potential predator taxa sampled by dip-net at each survey as fixed factors. The number of predators was assessed following available information of the feeding ecology of freshwater invertebrates (Ghetti, 1997; Tachet, 2010; Elliot & Dobson, 2015). As predator taxa for *Niphargus*, we considered the leeches of the genera *Glossiphonia* Johnson, 1816 and *Erpobdella* Blainville, 1818, the larvae of all genera of Odonata, all species of the coleopteran families Dytiscidae and Gyrinidae, larvae of the trichopteran family

Rhyacophilidae, the larvae of Neuroptera (formerly known as Planipennia) and the triclad flatworms *Dendrocoelum lacteum* (Müller, 1774) and *Polycelis nigra* (Müller, 1774). Finally, we included the year of survey, the site and the transect as random factors.

GLMMs were run in the R environment (R Development Core Team, 2018) using the package glmmTMB (Brooks et al., 2017).

RESULTS

Considering the total of transects and samplings, we recorded on average (± SE) 1.4 ± 0.49 individuals of *Niphargus* sp. per sampling. On average we recorded only 0.4 ± 0.17 individuals per visual sampling. On average (± SE) we recorded an abundance of 1.25 ± 0.34 individuals of potential predator taxa for *Niphargus* sp. per net sampling.

We detected a difference between day and night, in the number of *Niphargus* sp.; during the night, the number of individuals was significantly higher (table I, fig. 3).

GLMMs analysis revealed also that the observed abundance significantly relied on the sampling method used: dip netting allowed to detect more individuals than mere visual inspection (table I).

DISCUSSION

Our results show that in spring habitats the abundance of *Niphargus* sp. varies between night and day, regardless of the number of potential predators occurring at the springs' surfaces. Particularly the number of *Niphargus* amphipods is significantly higher during the night. The fact that populations living in groundwaters at the interface with surface waters in spring environments can more or less occasionally exploit epigean environments, is frequently reported for freshwater habitats, including springs and streams (Fišer et al., 2007; Manenti & Pezzoli, 2019). Surface habitats are richer in food, while subterranean habitats are slightly less used by potential predators, especially those using visual systems of prey detection, which generally are more active during the day (Thompson & Kiauta, 1994; Ghia et al., 2009; Manenti et al., 2013a). Depigmentation in freshwater organisms is usually considered as an adaptation to murky or subterranean waters (Galassi, 2001; Romero, 2020; Wagner, 2020). For stygobionts, the evolution of depigmentation is considered as an irreversible feature (Pipan & Culver, 2012; Culver & Pipan, 2014; Fišer et al., 2016); our observations suggest that when dwelling at the interface with surface environments, stygobionts are able to exploit these habitats effectively under the favourable conditions of darkness. Especially for crustaceans of the genus *Niphargus*, the fact of sheltering underground during the day and foraging outside at night may represent an important advantage, as in this scenario they can use the resources occurring in both environments. During the night more individuals cross the border between groundwaters and surface fresh waters to exploit the resources available in the surface layer; for various *Niphargus* species it has already been shown that drift is limited or does not occur during daytime (Kureck, 1967), with evidence of the ability of photoreception (Borowsky, 2011; Fišer et al., 2016). The advantages of photoreception may lie not only in avoiding surface habitats when these are riskier than groundwaters (Borowsky, 2011; Fišer et al., 2016), but also in exploiting them when conditions are safer, such as during the night.

Generally, in surface environments, nocturnal activity in both vertebrates and invertebrates is considered as an adaptive strategy to minimize the risk of predation (Huhta et al., 2000; Kotler et al., 2010) and is often supported by an upgraded sensitivity of the non-visual senses, that allow detection of threats under conditions of darkness (Vestheim et al., 2013; Bleicher et al., 2019). In our system, the fact that a stygobiont species without pigmentation showed a more pronounced nocturnal activity could be related to the necessity to avoid UV radiation during daytime and to the possibility to exploit a richer environment during the night when UV constraints are absent. Several studies have been developed on the different strategies that especially invertebrates have adopted to reduce the risk of damage caused by UV radiation (Jacobs et al., 2005; Block et al., 2009; Rudh & Qvarnstrom, 2013; Ciros-Perez et al., 2015). The most common ways that exist are avoidance behaviour, such as for example diel vertical migration in zooplankton, and photoprotective compounds, such as pigment formation (Gilbert & Hampton, 2001). In crustaceans, for example, photophobic behaviour is widespread, occurring both in depigmented stygobionts and in their pigmented surface relatives (Banta, 1910; Park et al., 1941; Ginet, 1960; Vandel, 1964; Fišer et al., 2016). The photophobic reaction observed in different *Niphargus* amphipods (Ginet, 1960; Vandel, 1964; Fišer et al., 2016) may be particularly useful for populations inhabiting spring habitats, for detecting when the daylight comes and thus being capable of avoiding exposition to UV radiation; this hypothesis could also be tested by further researches on *Niphargus* amphipods. In our study system, a limitation is linked to the fact that we were not able to identify the taxonomic status of the studied populations; although during the surveys we did not detect macroscopic differences between the observed individuals, the occurrence of multiple species cannot be excluded. Further researches on spring-dwelling populations of *Niphargus* species of certain identification should thus be performed in order to either confirm, or possibly modify our results.

In general, the nocturnal habits of the depigmented species suggest also that applying biotic indexes during the day in groundwater-fed springs, as well as in upwelling zones of streams and rivers, has some limitation. As depigmented species, normally living in groundwaters, likely exploit the surface habitats during the night, performing samplings of the community only during daytime may not be expected to provide exhaustive information in terms of species composition and abundance. Moreover, our results show that for benthic crustacean species the use of the dip-net increases the number of individuals collected if compared to simple, visual encounter surveys. In contrast, at the same sites, a recent study of ours on epigean species showed that for planarians the abundance of detected individuals is not different between visual counting surveys and dip-net samplings (Manenti & Barzaghi, 2020). The different pattern observed may be linked to the fact that planarians often occur on the surface of larger rocks and other elements of the substrate (Reynoldson & Young, 2000) where they can be easily observed, while *Niphargus* specimens, especially during day, shelter under or in the substrate.

The broad implication of the present research is, that the absence of light and fewer predators make an essential contribution in triggering the exploitation of spring habitats by amphipods of the genus *Niphargus*. As we have argued elsewhere, the study of diel activity in interface freshwater environments, specifically including both day- and night-time observations, may be considered a promising approach for understanding evolutionary and ecological patterns shaping the distribution of freshwater organisms.

ACKNOWLEDGEMENTS

We are grateful to F. Malarard, Z. Fiŝer, N. Anicic and L. Boscolo for help in retrieving old papers on *Niphargus*. We thank S. Ghioni and L. Vinci for the translation of German papers. The comments of J. C. von Vaupel Klein and of two anonymous reviewers increased the quality of this paper.

REFERENCES

ALFARO, C. & M. WALLACE, 1994. Origin and classification of springs and historical review with current applications. Environ. Geol., **24**: 112-124.

BALDERACCHI, M., A. PEREGO, G. LAZZARI, R. MUNOZ-CARPENA, M. ACUTIS, A. LAINI, A. GIUSSANI, M. SANNA, D. KANE & M. TREVISAN, 2016. Avoiding social traps in the ecosystem stewardship: the Italian fontanile lowland spring. Sci. Total. Environ., **539**: 526-535.

BANTA, A. M., 1910. A comparison of the reactions of a species of surface isopod with those of a subterranean species. Journ. Exp. Zool., **8**: 243-310.

BARKER, R. J., M. R. SCHOFIELD, W. A. LINK & J. R. SAUER, 2017. On the reliability of N-Mixture models for count data. Biometrics, **74**: 369-377.

BARZAGHI, B., G. F. FICETOLA, R. PENNATI & R. MANENTI, 2017. Biphasic predators provide biomass subsidies in small freshwater habitats: A case study of spring and cave pools. Freshw. Biol., **62**: 1637-1644.

BLEICHER, S. S., H. MARKO, D. J. MORIN, K. TEEMU & Y. HANNU, 2019. Balancing food, activity and the dangers of sunlit nights. Behav. Ecol. Sociobiol., **73**: 95.

BLOCK, W., R. I. L. SMITH & A. D. KENNEDY, 2009. Strategies of survival and resource exploitation in the Antarctic fellfield ecosystem. Biol. Rev., **84**: 449-484.

BLUME, J., E. BÜNNING & E. GÜNZLER, 1962. Zur Aktivitätsperiodik bei Höhlentieren. Naturwissenschaften, **49**: 525-525.

BOROWSKY, B., 2011. Responses to light in two eyeless cave dwelling amphipods (*Niphargus ictus* and *Niphargus frasassianus*). J. Crust. Biol., **31**: 613-616.

BOTTAZZI, E., M. C. BRUNO, M. MAZZINI, V. PIERI & G. ROSSETTI, 2008. First report on Copepoda and Ostracoda (Crustacea) from northern Apenninic springs (N. Italy): a faunal and biogeographical account. J. Limnol., **67**: 56-63.

BRESSI, N., M. ALJANCIC & L. LAPINI, 1999. Notes on presence and feeding of *Proteus anguinus* Laurenti, 1768 outside caves. Riv. Idrobiol., **38**: 431-435.

BROOKS, M. E., K. KRISTENSEN, K. J. VAN BENTHEM, A. MAGNUSSON, C. W. BERG, A. NIELSENN, H. J. SKAUG, M. MAECHLER & B. BOLKER, 2017. glmmTMB balances speed and flexibility among packages for zero-inflated Generalized Linear Mixed Modeling. R Journal, **9**: 378-400.

CANTONATI, M., R. GERECKE & E. BERTUZZI, 2006. Springs of the Alps – sensitive ecosystems to environmental change: from biodiversity assessments to long-term studies. Hydrobiologia, **562**: 59-96.

CANTONATI, M., R. GERECKE, I. JUTTNER & E. J. COX, 2011. Springs: neglected key habitats for biodiversity conservation – Introduction to the Special Issue. J. Limnol., **70**: 1.

CANTONATI, M., S. POIKANE, C. M. PRINGLE, L. E. STEVENS, E. TURAK, J. HEINO, J. S. RICHARDSON, R. BOLPAGNI, A. BORRINI, N. CID, M. CTVRTLIKOVA, D. M. P. GALASSI, M. HAJEK, I. HAWES, Z. LEVKOV, L. NASELLI-FLORES, A. A. SABER, M. DI CICCO, B. FIASCA, P. B. HAMILTON, J. KUBACKA, S. SEGADELLI & P. ZNACHOR, 2020. Characteristics, main impacts, and stewardship of natural and artificial freshwater environments: consequences for biodiversity conservation. Water, **12**: 260.

CAO, Y., A. W. BARK & W. P. WILLIAMS, 1997. Analysing benthic macroinvertebrate community changes along a pollution gradient: A framework for the development of biotic indices. Water Res., **31**: 884-892.

CEREGHINO, R. & P. LAVANDIER, 1998. Influence of hydropeaking on the distribution and larval development of the Plecoptera from a mountain stream. Regul. River, **14**: 297-309.

CIROS-PEREZ, J., E. ORTEGA-MAYAGOITIA & J. ALCOCER, 2015. The role of ecophysiological and behavioral traits in structuring the zooplankton assemblage in a deep, oligotrophic, tropical lake. Limnol. Oceanogr., **60**: 2158-2172.

COLLIER, K. J., P. K. PROBERT & M. JEFFRIE, 2016. Conservation of aquatic invertebrates: concerns, challenges and conundrums. Aquat. Conserv., **26**: 817-837.

COPILAS-CIOCIANU, D., C. FISER, P. BORZA, G. BALAZS, D. ANGYAL & A. PETRUSEK, 2017. Low intraspecific genetic divergence and weak niche differentiation despite wide ranges and extensive sympatry in two epigean *Niphargus* species (Crustacea: Amphipoda). Zool. J. Linn. Soc., Lond., **181**: 485-499.

CULVER, D. C., J. R. HOLSINGER & D. J. FELLER, 2012. The fauna of seepage springs and other shallow subterranean habitats in the Mid-Atlantic piedmont and coastal plain. Northeast. Nat., **19**: 1-42.

CULVER, D. C. & T. PIPAN, 2014. Shallow subterranean habitats: ecology, evolution, and conservation: 1-258. (Oxford University Press, New York, NY).

CULVER, D. C. & T. PIPAN, 2019. The biology of caves and other subterranean habitats (2nd ed.): 1-254. (Oxford University Press, New York, NY).

DANIELOPOL, D. L., R. ROUCH & C. BOU, 1999. High Amphipoda species richness in the Nert groundwater system (southern France). Crustaceana, **72**: 863-882.

DE LUCA, D. A., M. LASAGNA & L. DEBERNARDI, 2020. Hydrogeology of the western Po plain (Piedmont, NW Italy). J. Maps, **16**: 265-273.

DHOMPS-AVENAS, M. & J. MATHIEU, 1983. Étude d'une population épigée de l'Amphipode souterrain *Niphargus rhenorhodanensis:* réponse physiologique face aux conditions hydrologiques du milieu. Vie et Milieu, **33**: 119-125.

DI SABATINO, A., B. CICOLANI & R. GERECKE, 2003. Biodiversity and distribution of water mites (Acari, Hydrachnidia) in spring habitats. Freshw. Biol., **48**: 2163-2173.

DODDS, W. K., L. BRUCKERHOFF, D. BATZER, A. SCHECHNER, C. PENNOCK, E. RENNER, F. TROMBONI, K. BIGHAM & S. GRIEGER, 2019. The freshwater biome gradient framework: predicting macroscale properties based on latitude, altitude, and precipitation. Ecosphere, **10**: e02786.

ELLIOTT, J. M., 2002. A quantitative study of day-night changes in the spatial distribution of insects in a stony stream. J. Anim. Ecol., **71**: 112-122.

ELLIOT, J. M. & M. DOBSON, 2015. Freshwater leeches of Britain and Ireland: 1-108. (Freshwater Biological Association, Sudbury).

FIŠER, C., R. KEBER, V. KEREZI, A. MOSKRIC, A. PALANDANCIC, V. PETKOVSKA, H. POTOCNIK & B. SKET, 2007. Coexistence of species of two amphipod genera: *Niphargus timavi* (Niphargidae) and *Gammarus fossarum* (Gammaridae). J. Nat. Hist., **41**: 2641-2651.

FIŠER, Z., L. NOVAK, R. LUSTRIK & C. FISER, 2016. Light triggers habitat choice of eyeless subterranean but not of eyed surface amphipods. Sci. Nat., Heidelberg, **103**: 7.

FLORENCIO, M., C. DIAZ-PANIAGUA, I. GOMEZ-MESTRE & L. SERRANO, 2012. Sampling macroinvertebrates in a temporary pond: comparing the suitability of two techniques to detect richness, spatial segregation and diel activity. Hydrobiologia, **689**: 121-130.

GALASSI, D. M. P., 2001. Groundwater copepods (Crustacea: Copepoda): diversity patterns over ecological and evolutionary scales. Hydrobiologia, **453**: 227-253.

GHETTI, P. F, 1997. Indice Biotico Esteso (I.B.E.): Manuale di applicazione: 1-222. (Provincia Autonoma di Trento, Trento).

GHIA, D., G. FEA, M. SPAIRANI, F. BERNINI & P. A. NARDI, 2009. Movement behaviour and shelter choice of the native crayfish *Austropotamobius pallipes* complex: survey on a population in a semi-natural pond in northern Italy. Mar. Freshw. Behav. Phys., **42**: 167-185.

GILBERT, J. D., I. DE VICENTE, F. ORTEGA, R. JIMENEZ-MELERO, G. PARRA & F. GUERRERO, 2015. A comprehensive evaluation of the crustacean assemblages in southern Iberian Mediterranean wetlands. J. Limnol., **74**: 169-181.

GILBERT, J. J. & S. E. HAMPTON, 2001. Diel vertical migrations of zooplankton in a shallow, fishless pond: a possible avoidance-response cascade induced by notonectids. Freshw. Biol., **46**: 611-621.

GINET, R., 1960. Ecologie, éthologie et biologie de "*Niphargus*" (Amphipodes Gammaridés hypogés). Ann. Speleol., **15**: 127 -237.

HORTON, T., J. LOWRY, C. DE BROYER, D. BELLAN-SANTINI, C. O. COLEMAN, L. CORBARI, M. J. COSTELLO, M. DANELIYA, J. DAUVIN, C. FISER, R. GASCA, M. GRABOWSKI, J. M. GUERRA-GARCIA, E. HENDRYCKS, L. HUGHES, D. JAUME, K. JAZDZEWSKI, Y. KIM, R. KING, T. KRAPP-SCHICKEL, S. LECROY, A.-N. LÖRZ, T. MAMOS, A. R. SENNA, C. SEREJO, B. SKET, J. F. SOUZA-FILHO, A. H. TANDBERG, J. D. THOMAS, M. THURSTON, W. VADER, R. VÄINÖLÄ, R. VONK, K. WHITE & W. ZEIDLER, 2019. World Amphipoda database. [Accessed at: http://www.marinespecies.org/amphipoda on 2021-01-14.] doi: 10.14284/368

HUDEC, I., C. FISER & J. DOLANSKY, 2017. *Niphargus diadematus* sp. n. (Crustacea, Amphipoda, Niphargidae), an inhabitant of a shallow subterranean habitat in South Moravia (Czech Republic). Zootaxa, **4291**: 41-60.

HUHTA, A., T. MUOTKA & P. TIKKANEN, 2000. Nocturnal drift of mayfly nymphs as a post-contact antipredator mechanism. Freshw. Biol., **45**: 33-42.

JACOBS, J. L., T. L. CARROLL & G. W. SUNDIN, 2005. The role of pigmentation, ultraviolet radiation tolerance, and leaf colonization strategies in the epiphytic survival of phyllosphere bacteria. Microb. Ecol., **49**: 104-113.

KARAMAN, G., 1975. Contribution to the knowledge of the Amphipoda. Three *Niphargus* species from Yugoslavia and Italy, *N. ambulator* n. sp., *N. pupetta* (Sket) and *N. transitivus* Sket (fam. Gammaridae). Poljoprivreda i šumarstvo, **21**: 13-34.

KOPERSKI, P., 2011. Diversity of freshwater macrobenthos and its use in biological assessment: a critical review of current applications. Environ. Rev., **19**: 16-31.

KOTLER, B. P., J. BROWN, S. MUKHERJEE, O. BERGER-TAL & A. BOUSKILA, 2010. Moonlight avoidance in gerbils reveals a sophisticated interplay among time allocation, vigilance and state-dependent foraging. Proc. Royal Soc., (B) **277**: 1469-1474.

KURECK, A., 1967. Über die tagesperiodische Ausdrift von *Niphargus aquilex schellenbergi* Karaman aus Quellen. Zeitschrift für Morphologie und Ökologie der Tiere, **58**: 247-262.

KUSANO, H. & T. KUSANO, 1991. Diel activity of breeding individuals of a fresh-water amphipod, *Jesogammarus spinopalpus*. J. Ethol., **9**: 105-111.

LEUNDA, P. M., J. OSCOZ, R. MIRANDA & A. H. ARINO, 2009. Longitudinal and seasonal variation of the benthic macroinvertebrate community and biotic indices in an undisturbed Pyrenean river. Ecol. Indic., **9**: 52-63.

MACAVOY, S. E., A. BRACISZEWSKI, E. TENGI & D. W. FONG, 2016. Trophic plasticity among spring vs. cave populations of *Gammarus minus*: examining functional niches using stable isotopes and C/N ratios. Ecol. Res., **31**: 589-595.

MALARD, F., M. J. DOLE-OLIVIER J. MATHIEU & F. STOCH, 2002. Sampling manual for the assessment of regional groundwater biodiversity: 1-111. (PASCALIS Project, Lyon).

MANENTI, R. & B. BARZAGHI, 2020. Is landscape of fear of macroinvertebrate communities a major determinant of mesopredator and prey activity? Knowl. Manag. Aquat. Ecosyst., **421**: 8.

MANENTI, R., M. DENOËL & G. F. FICETOLA, 2013a. Foraging plasticity favours adaptation to new habitats in fire salamanders. Anim. Behav., **86**: 375-382.

MANENTI, R., D. GHIA, G. FEA, G. F. FICETOLA, E. PADOA-SCHIOPPA & C. CANEDOLI, 2019. Causes and consequences of crayfish extinction: Stream connectivity, habitat changes, alien species and ecosystem services. Freshw. Biol., **64**: 284-293.

MANENTI, R., R. PENNATI & G. F. FICETOLA, 2015. Role of density and resource competition in determining aggressive behaviour in salamanders. J. Zool., **296**: 270-277.

MANENTI, R. & E. PEZZOLI, 2019. Think of what lies below, not only of what is visible above, or: a comprehensive zoological study of invertebrate communities of spring habitats. Eur. Zool. J., **86**: 272-279.

MANENTI, R., M. E. SIESA & G. F. FICETOLA, 2013b. Odonata occurence in caves: active or accidentals? A new case study. J. Cave Karst Stud., **75**: 205-209.

MARKOVIĆ, V., B. NOVAKOVIĆ, M. ILIĆ & V. NIKOLIĆ, 2018. Epigean niphargids in Serbia: New records of *Niphargus valachicus* Dobreanu & Manolache, 1933 (Amphipoda: Niphargidae), with notes on its ecological preferences. Acta Zool. Bulg., **70**: 45-50.

MATHIEU, J., K. ESSAFI & H. CHERGUI, 1999. Spatial and temporal variations of stygobite amphipod populations in interstitial aquatic habitats of karst floodplain interfaces in France and Morocco. Ann. Limnol. – Int. J. Limnol., **35**: 133-139.

MATHIEU, J., K. ESSAFICHERGUI & F. JEANNEROD, 1994. A gradient of interstitial *Niphargus rhenorhodanensis* populations in 2 karst floodplain transition zones of the French Jura. Hydrobiologia, **286**: 129-137.

MORA, M. A., W. E. GRANT, L. WILKINS & H. H. WANG, 2013. Simulated effects of reduced spring flow from the Edwards Aquifer on population size of the fountain darter (*Etheostoma fonticola*). Ecol. Model., **250**: 235-243.

MORI, N., T. KANDUC, M. OPALICKI SLABE & A. BRANCELJ, 2015. Groundwater drift as a tracer for identifying sources of spring discharge. Groundwater, **53**: 123-132.

MÜLLER, K., A. KURECK & A. MÜLLER-HAECKEL, 1963. Zur Tagesperiodik von *Niphargus aquilex schellenbergi* Karaman (Gammaridae, Amphipoda). Naturwissenschaften, **50**: 579-580.

MUSONGE, P. S. L., P. BOETS, K. LOCK, N. M. D. AMBARITA, M. A. E. FORIO, D. VERSCHUREN

& P. L. M. Goethals, 2019. Baseline assessment of benthic macroinvertebrate community structure and ecological water quality in Rwenzori rivers (Albertine rift valley, Uganda) using biotic-index tools. Limnologica, **75**: 1-10.

Niemiller, M. L., B. M. Fitzpatrick & B. T. Miller, 2008. Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: *Gyrinophilus*) inferred from gene genealogies. Mol. Ecol., **17**: 2258-2275.

Niemiller, M. L., M. S. Osbourn, D. B. Fenolio, T. K. Pauley, B. T. Miller & J. R. Holsinger, 2010. Conservation status and habitat use of the West Virginia spring salamander (*Gyrinophilus subterraneus*) and spring salamander (*G. porphyriticus*) in General Davis Cave, Greenbrier Co., West Virginia. Herp. Cons. Biol., **5**: 32-43.

Oberrisser, P. & J. Waringer, 2011. Larval salamanders and diel drift patterns of aquatic invertebrates in an Austrian stream. Freshw. Biol., **56**: 1147-1159.

Park, O., T. Roberts & S. Harris, 1941. Preliminary analysis of activity of the cave crayfish, *Cambarus pellucidus*. Am. Nat., **75**: 154-171.

Pezzoli, E., 2010. Notes on new or rare taxa of crustaceans and molluscs from a “fontanile” in Arzago d'Adda, Bergamo, Italy (Crustacea, Mollusca). Biodiv. J., **1**: 45-55.

Pipan, T. & D. C. Culver, 2012. Convergence and divergence in the subterranean realm: a reassessment. Biol. J. Linn. Soc., Lond., **107**: 1-14.

R Development Core Team, 2018. R: A language and environment for statistical computing: https://www.R-project.org. (R Foundation for Statistical Computing, Vienna).

Reynoldson, J. D. & J. O. Young, 2000. A key to the freshwater triclads of Britain and Ireland with notes on their ecology: 1-72. (Freshwater Biological Association, Ambleside, Cumbria).

Rincon, P. A. & J. Lobon Cervia, 1997. Temporal patterns in macroinvertebrate drift in a northern Spanish stream. Mar. Freshwater Res., **48**: 455-464.

ROBERTSON, A. L., J. W. N. SMITH, T. JOHNS & G. S. PROUDLOVE, 2009. The distribution and diversity of stygobites in Great Britain: an analysis to inform groundwater management. Q. J. Eng. Geol. Hydroge., **42**: 359-368.

ROMERO, A., 2009. Cave biology: 1-291. (Cambridge University Press, New York, NY).

ROMERO, A., 2020. Hypogean communities as cybernetic systems. Diversity, **12**: 413.

RUDH, A. & A. QVARNSTROM, 2013. Adaptive colouration in amphibians. Semin. Cell Dev. Biol.., **24**: 553-561.

SMITH, A. J., R. W. BODE & G. S. KLEPPEL, 2007. A nutrient biotic index (NBI) for use with benthic macroinvertebrate communities. Ecol. Indic., **7**: 371-386.

STOCH, F., 2000. Isopodi ed anfipodi (Crustacea, Malacostraca) della Provincia di Bergamo: note sulle specie rinvenute nelle grotte e nelle sorgenti. In: E. PEZZOLI & F. SPELTA (eds.), I molluschi delle sorgenti e delle 'Acque sotterranee', IX. Aggiornamento al censimento: 231-241. (Monogr. Nat. Bresc., Brescia).

TACHET, H., 2010. Invertébrés d'eau douce : Systématique, biologie, écologie: 1-587. (CNRS, Paris).

THOMPSON, D. J. & B. KIAUTA, 1994. Odonatospeleology: dragonflies in caves, with a checklist of the known records. Opusc. Zool. Flumin., **118**: 1-10.

VANDEL, A., 1920. Sur la faune des sources. Bull. Soc. Zool. Fr., **45**: 177-183.

VANDEL, A., 1964. Biospeleologie: la biologie des animaux cavernicoles: 1-619. (Gauthiers-Villars, Paris).

VESTHEIM, H., S. BRUCET & S. KAARTVEDT, 2013. Vertical distribution, feeding and vulnerability to tactile predation in *Metridia longa* (Copepoda, Calanoida). Mar. Biol. Res., **9**: 949-957.

VON FUMETTI, S. & P. NAGEL, 2011. A first approach to a faunistic crenon typology based on functional feeding groups. J. Limnol., **70**: 147-154.

WAGNER, H. P., 2020. Stygofauna of Oman, 8*). The Thermosbaenacea collected during the biological groundwater survey of Oman of 1996. Crustaceana, **93**: 1197-1232.

WHITE, W. B., 2019. Springs. In: W. B. WHITE, D. C. CULVER & T. PIPAN (eds.), Encyclopedia of caves: 1031-1040. (Academic Press, Cambridge, MA).

First received 7 January 2021.

Final version accepted 16 February 2021.

[CAPTIONS]

Fig. 1. Diagram of a “fontanile” spring underlining differences occurring between surface and groundwater habitats during the day. “Fontanili” springs are historically managed by humans through insertion of pipes in the substrate to facilitate water gushing. White silhouettes represent *Niphargus* sp. amphipods. Black silhouettes represent potential predators (dragonfly larvae). [Drawn modified from Andrea Melotto (unpubl.]

Fig. 2. Location of the study sites. The yellow circle and the red box identify the general location of the study area. The orange line shows the northern border of the “fontanili” range. The exact locations of the sites are represented by blue circles.

Fig. 3. Boxplot of the relationships between the numbers of individuals of *Niphargus* sp., the moment of the day on which surveys have been performed, and the methods of survey.

TABLE I

Results of the GLMMs analysis. In **bold** the significant results that show the relationship between the number of specimens of *Niphargus* sp. and the three fixed factors considered in the analysis: day/night period, number of predators, and visual observation/dip-netting. The column "Estimate" provides the values of the estimated regression parameters; "z" reports the value of the z-scores

Variable	Estimate	z	*P*
Period (night-time)	1.39	2.68	< **0.01**
Number of predators	0.91	0.81	0.60
Sampling method (dip-netting)	1.12	3.24	**0.01**

[Basically, the Supplementary Table I, provided separately in Excel, is meant for online publication only, but the choice is, of course, with BRILL. JCvVK]

SUPPLEMENTARY TABLE I

Data used for the GLMMs analysis performed to assess the relationship between the number of *Niphargus* sp. and the moment of observation (day/night), the sampling method (visual/net) and the number of potential predators. The moment of observation is provided in the column "Night" where 1 stays for night-time and 0 for daytime. The sampling method is provided in the column "Sampling method (Net)", where 1 stays for dip-netting samplings and 0 for visual surveys. The column "Value_predator_used for analysis" reports the number of potential predator taxa recorded at each sampling, which was log-transformed prior to analysis