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Resistance of groundwater invertebrates to droughts: Two new cases in planarians and isopods

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Abstract

Benthic invertebrate communities are deeply influenced by hydrological variability in surface freshwater habitats. However, the responses of macroinvertebrates composing stygofauna in aquifers and unsaturated groundwater environments are not well understood nor specifically studied. Here, we present two new cases of resistance to drying in groundwater-dwelling animals: planarians of the genus *Dendrocoelum* and the isopod crustacean *Monolistra pavani*. Multiple surveys were performed in 53 caves of the Italian Alps and Apennines, sampling different stable and unstable subterranean aquatic environments. Planarians and crustaceans were searched visually and by distressing the substrate. When we detected individuals in dry sectors, we photographed them, observed their features, and released them in pools or streams of the same cave. We rinsed dry individuals of *M. pavani* in small tanks and recorded the time of reactivation. In a dried subterranean stream, we observed a *Dendrocoelum* planarian encapsulated in a thick mucus layer, which after a total of 5 min started gliding slowly and reached a length of 18 mm. During the drought of January–April 2022, we detected several individuals of *M. pavani* in dry areas of two caves. In total, 72% of the tested individuals were able to reactivate. This percentage decreased to 42% after 20 days of dryness. Time to reactivate ranged between 0 and 30 s. Living individuals were observed even after 39 days of drought. Our observations provide new insights into the natural history of groundwater-dwelling invertebrate taxa that are often neglected in the studies of freshwater ecology. Resistance of groundwater-dwelling animals to droughts has several implications for global climatic changes and could be species- and site-specific, as previously observed in surface macroinvertebrates.

KEYWORDS
benthos, cave, desiccation, dry, freshwater, groundwater

INTRODUCTION

Several karst areas in the Mediterranean basin are experiencing a substantial reduction in average rainfall over the last decade. Climate change and infiltration can reduce aquifer recharge in karst and non-karst areas, thus impacting groundwater storage. Groundwaters are fragile and understudied ecosystems, which host organisms with unique features and peculiar specializations that evolved to live underground.

While microclimatic conditions are relatively stable, water flow in aquifers can be dynamic, especially in karst areas where circulation can be quickly affected by surface conditions such as heavy rains (Dumnicka et al., 2017; Galassi et al., 2009). Benthic invertebrate communities are heavily influenced by hydrological variability in freshwater habitats. For instance, an adaptation of surface freshwater planarians to irregular hydroperiods of Mediterranean watercourses is the ability to encyst themselves inside mucus capsules when droughts occur (Vila-Farré et al., 2011). Groundwater-dwelling invertebrates have been observed in subterranean sites at the interface between saturated and vadose zones (Culver & Pipan, 2014). For example, the planarian *Atrioplanaria morisii*, a stenoendemic species known found exclusively in a locality in northwestern Italy, inhabits a cave that is flooded during the wet seasons (spring and autumn) and is completely dry during winter and summer (Manenti et al., 2018). Even if it is possible that exceptional drying periods are related to local extinctions (and successive recolonizations through migration from the saturated zone) in sites at the interface with the vadose zone, adaptations to resist dryness are likely to have been developed by groundwater-dwelling animals. However, the responses of macroinvertebrates composing stygofauna in aquifers and unsaturated groundwater environments are not well understood nor extensively studied. To the best of our knowledge, few observations of resistance to dryness in groundwater have been reported in the literature. They involve the subterranean amphipod crustaceans of the genus *Niphargus* (Ginet, 1960; Vandel, 1964). This organism can survive up to 11 months in small, wet burrows on the dry substrate (clay) and then resume normal activity when the water level rises (Ginet, 1960). Also, amphipods of the genus *Crangonyx* and *Stygobromus*, which occur in epikarst drip pools and hypotelminorheic (seep) habitats, have shown, during experimental drying trials, the ability to burrow and survive for at least 4 weeks (Gilbert et al., 2018).

Here, we present two new cases of resistance to dryness in groundwater-dwelling animals: planarians of the genus *Dendrocoelum* and the isopod crustacean *Monolistra pavani*.

The genus *Dendrocoelum* (Figure 1) represents free-living flatworms with many species in groundwater, where they play important ecological roles (Barzaghi et al., 2021). While the currently known subterranean species of the genus *Dendrocoelum* in Italy seem to have quite narrow ranges (Manenti et al., 2018, 2019), recent observations suggest that many populations without a taxonomic status are spread in most aquifers of the peninsula (Manenti et al., 2016, 2020). *M. pavani* is an aquatic species (Figure 2a) described in a subterranean system in Northern Italy in the Como district as well as the caves located between Lombardy and Switzerland (Vuilleumier, 1973).

MATERIALS AND METHODS

We conducted multiple surveys from October 2016 to April 2022 in 53 caves of the Italian Alps and Apennines. Three caves comprise the whole and deep subterranean system forming the locality type of *M. pavani*, which is in the surroundings of Erba (Como district, Lombardy). Five caves are localities at the border between Lombardy and Switzerland with both epikarst streams and aquifers where western populations currently attributed

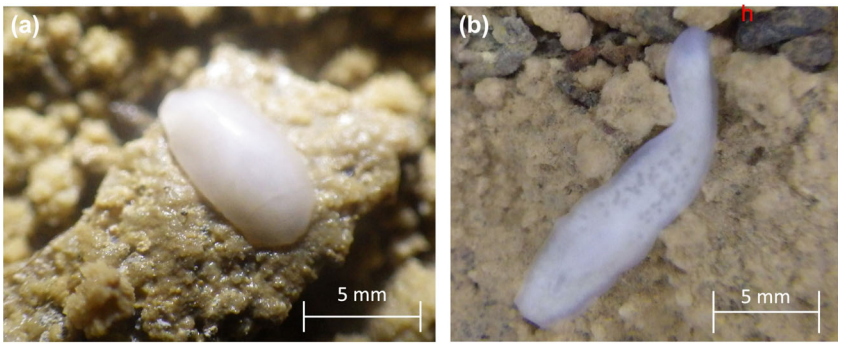

FIGURE 1 *Dendrocoelum* sp. planarians recorded in the cave "Tanna da Scaggia" in Liguria (northwestern Italy). (a) A dry individual found after the drought of part of the subterranean stream. (b) The same individual photographed in (b), after reactivation in water; h, head.

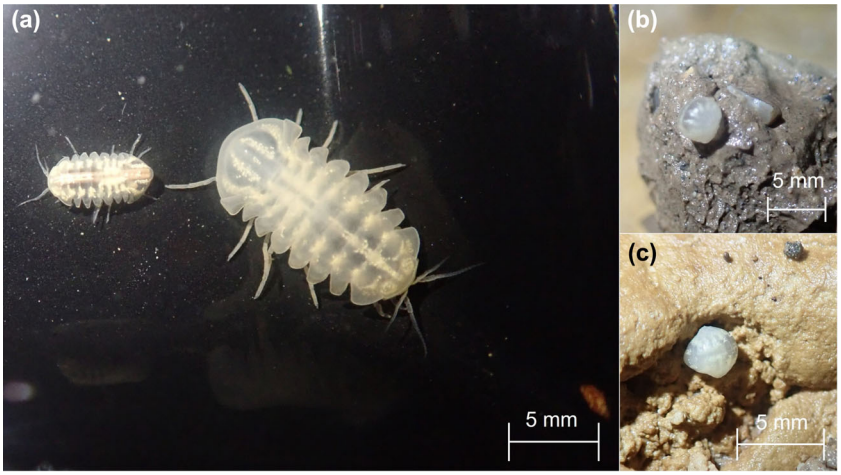

FIGURE 2 The isopod *Monolistra pavani*. (a) Female (left) and male (right) adult individuals. (b, c) Dry individuals rolled up on different substrates.

to *M. pavani* occur. All the other caves are sites with subterranean hydrographic systems in which the occurrence of histologically identified individuals of *Dendrocoelum* sp. planarians has been observed at least once (Barzaghi et al., unpublished). We entirely explored all the caves that were compatible with our speleological equipment, focusing on all the freshwater habitats (streams, siphons, creeks, drip pools, dripping layers on the walls).

Planarians and crustaceans were searched visually and by distressing the substrate of each groundwater habitat. When we detected the organisms in dry watercourses, we photographed them, recorded their features, and released them in pools or streams of the same cave. After the first finding of dry individuals of *Monolistra* crustaceans in February 2022, we performed five further surveys in the same sites untill April 2022. Prior to release, the animals were rinsed in a small Petri dish with 10 ml of tap water, and we recorded the time required to reactivate and the percentage of individuals reactivated after a maximum time of 20 min. For every individual, we annotated the last date in which the pools of occurrence were not dry. In case of dried couples (*M. pavani* individuals often occur in couples with males that hold on to females for weeks), we did not split them, and we recorded the time of reactivation of both individuals. Moreover, we recorded the temperature and the relative humidity (UR %) of the air and the substrate using two DS1923-F5# Hygrochron Temperature and Humidity Data Logger and an HTI-350 Hygrometer. We used a generalized mixed model and a linear mixed model to assess the relationship between the reactivation rate and reactivation time, respectively, with the calculated time of dryness and the sex of the animals. The cave site was considered a random factor.

RESULTS

When surveying the cave "Tanna da Scaggia" in Liguria on 3 January 2017, we found one planarian that we attributed without histological analysis to the genus *Dendrocoelum* in a dried area of the cave with a slow-flowing subterranean stream. The planarian was motionless on a small stone covered in a thick mucus layer (Figure 1a). The total length was about 9 mm. We removed the individual and placed it in a pool located 25 m deeper in the cave. After 2 min, the individual started moving its head and protracting its body. After 5 min, it started gliding slowly and reached a length of 18 mm (Figure 1b).

During the drought of January–April 2022, we detected several individuals of *M. pavani* in dry areas of the cave LoCo 2208 in Lombardy and in the cave "Buco della Sovaglia" (Rovio, Switzerland), while in the other caves that we examined, we did not find dry individuals. All the individuals found in dry areas were rolled up and completely motionless (Figure 2b,c). In total, 72% of the tested individuals were able to reactivate (Figure 2c). This percentage decreased to 42% after 20 days of dryness. Time to reactivate ranged between 0 and 30 s. Reactivation was negatively correlated to the estimated time of dryness ($\chi^2 = 18.58$; $p < 0.001$) but not with sex ($\chi^2 = 1.04$; $p = 0.3$). Individuals that experienced longer dryness took significantly longer to reactivate ($F = 7.62$; $p < 0.01$). In the cave, LoCo 2208 substrate was mainly composed of gravels, sand, and soft deposits; UR of air was 93% at 1 m of height and 94.4% at the substrate level, with an air temperature of 9.6°C. In this site, an area that was dry on 16 February 2022 was flooded on 4 March 2022 and became dry again after 5 days. After the flood, we observed live and active *M. pavani* individuals that

rolled themselves up again when their environment started drying. They were able to reactivate during successive tests performed on 16 March 2022. Many couples and two females with eggs were able to reactivate.

After 39 days of drought in Sovaglia cave, we detected a live *M. pavani* individual among both dead and rolled-up individuals sheltered in dry crevices (UR: 76%, *T*: 11.4°C). Few tiny pools with limited depth still occurred and hosted other live individuals.

DISCUSSION

Our observations of stygobionts displaying resistance to dryness in underground watercourses have important implications as follows:

1. They represent two distinct invertebrate genera with ability to survive droughts affecting groundwater. The occurrence of viable individuals surviving relatively long periods of drought suggests that the harsh impacts of drought can be overcome by resistance and successive recovery of stygofauna communities.
2. Resistance of groundwater-dwelling animals could be species- and site-specific, as already observed in surface macroinvertebrates (Durkota et al., 2019). Substrate features allowing humidity retention and morphological diversity of subterranean streams may improve survival. Moreover, the resistance of stygobionts seems possible during prolonged droughts.
3. Our observations provide new insights into the natural history of groundwater-dwelling invertebrate taxa that are often neglected in the studies of freshwater ecology. Resistance in planarians of the genus *Dendrocoelum* is here reported for the first time. It suggests that some responses to desiccation could have evolved in these planarians and call for further studies. The only other observations of drought resistance in subterranean habitats come from the European groundwater-dwelling planarian *Atrioplanaria notadena* (Ginet & Puglisi, 1964) both in laboratory conditions and by artificially flooding the substrate of cave-observed planarians surviving droughts. In the laboratory, they curled into clay burrows that provided enough humidity to survive. The possibility of resistance to droughts has also been attributed to the cave-dwelling and amphibious planarian *Amphibioplana onnisi* (Stocchino et al., 2021), for which ecological studies, however, are required. In aquatic isopods, droughts are considered one of the most important factors driving species distribution (Styron, 1968). In *Monolistra* crustaceans, rolling-up capability is extremely effective and is generally associated with defensive responses against predators (Manenti et al., 2020). Our findings show that this behavior is displayed in different adverse conditions and can last for relatively long periods of droughts.

We suggest that the following subjects need to be further investigated: (1) estimating how prolonged droughts on the surface are associated with a reduction in abundance and diversity of the stygofauna in groundwater; (2) evaluating the relative role of desiccation survival and migration from saturated areas in allowing stygofauna to recover at the interface with vadose zone; and (3) testing the existence of physiological adaptations among stygobiont species that facilitate survival in dry periods.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Manenti, 2022) are available from Figshare: https://doi.org/10.6084/m9.figshare.19747726.v1.

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