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CONSERVATION VALUE OF ARTIFICIAL SUBTERRANEAN SYSTEMS: A CASE STUDY IN AN ABANDONED MINE IN ITALY

ISAIA Marco[a], GIACHINO Pier Mauro[b], SAPINO Emanuele[a], CASALE Achille[c], BADINO Guido[a]

[a] Department of Animal and Human Biology, University of Turin. Via Accademia Albertina, 13 - 10123 Torino, Italy.

[b] Settore Fitosanitario Regionale. Environment Park, Palazzina A2, Via Livorno, 60 – 10144 Torino, Italy.

[c] Department of Zoology and Evolutionary Genetics, University of Sassari. Via Muroni, 25 – 07100 Sassari, Italy.

Communicating author:

Marco ISAIA, Department of Animal and Human Biology, University of Turin. Via Accademia Albertina, 13 - 10123 Torino, Italy. .Tel. +390116704544, Fax: +390116704508. E-mail: marco.isaia@unito.it

ABSTRACT

Subterranean ecosystems often harbour unique and specialized biocoenoses of considerable scientific interest and high potential conservation value. In view of the peculiar species assemblage in the abandoned tunnels of a talc mine complex in the north-western Italian Alps (Germanasca Valley, Province of Turin, NW Italy), the aims of the present work were (i) to investigate the subterranean invertebrate fauna, (ii) to assess the impact of tourism activities on the invertebrate fauna, and (iii) to assess the conservation value of the terrestrial invertebrate community and associated habitats. The study was carried out at four sites: one tunnel restored for tourism purposes, two abandoned tunnels and a wild cave. The results of several statistical analyses, including ANOVA, PCA and CCA, showed that the eutrophic conditions induced by past human activity could improve the quality of the subterranean habitat. On the other hand, a massive intervention for tourism purposes could seriously jeopardize the survival of the most sensitive species. The increased thermic instability and mean temperature had a significant negative effect on the local populations of cave-dwelling arthropods, whose ecological optimum is determined primarily by low thermic instability, cold temperatures and intermediate conditions of eutrophy. On the basis of our results we strongly recommend to avoid thermic isolation of any part of tunnels, as it is the primary factor affecting the most sensitive species.

Key Words: cave-dwelling arthropods, CCA, eutrophy, human disturbance, thermic instability

INTRODUCTION

Subterranean ecosystems are widely considered to be stable (Badino, 1995; Ferreira & Martins, 1998; Ward & Palmer, 1994), as they are light-deficient and thermically stable, with mean temperature values corresponding closely to the mean annual temperature outside the cave (Smithson, 1991). In the absence of light and primary producers, cave habitats are largely oligotrophic, receiving poor supplies of degradable organic matter (Engel at al., 2004; Humphreys, 1991; Hüppop, 2005) and relying almost exclusively on organic matter from surface (epigean) habitats (Poulson & Lavoie, 2000). Primary sources of energy are generally plant matter (Humphreys, 1991), guano (Hamilton-Smith, 2001) and carrion (Braack, 1989). As a result, when compared with surface ecosystems, subterranean cave environments often have a low abundance and diversity of organisms (Holsinger, 1998). These ecosystems often harbour unique and specialized biocoenoses of considerable scientific interest and high potential conservation value. The most diverse cave ecosystems reported (mostly in the tropics) are those with substantial inputs of externally derived organic energy sources, such as plant root material (Stone et al., 2005) and bat and cricket faeces (Ferreira & Martins, 1988).

In some cases, artificial subterranean networks, like abandoned mines, ancient underground fortresses or old military bunkers, can present the same environmental conditions as natural subterranean habitats: light deficiency, constant high relative humidity and thermic stability. One possible difference between artificial and natural subterranean habitats may involve trophic inputs, which may be abundant in abandoned mines on account of huge quantities of decaying wood deriving from human activity. A dramatic rise in trophic resources could significantly increase the carrying capacity of the system, acting as a source of nutrition for fungi and detritivores. Indeed, the faunal assemblages harboured by this kind of artificial ecosystem could be fully comparable to any natural one, with similar potential conservation value. An example is the military bunker of Vernante (CN, north-western Italy). This artificial subterranean system has been studied since the second half of the last century because of its peculiar faunal assemblage, characterized by the co-existence of 14 specialized subterranean taxa, with several elements precinctive to the bunker (Casale & Giachino, 2008).

Recreational uses of subterranean systems (natural or artificial) saw a marked increase in the 20th century (Cigna, 2005; Gunn et al., 2000). Some subterranean systems may receive few or no visitors in any particular year because they are remote, too short to be considered worthwhile, or because access is difficult or prohibited. Others, including "show caves" and restored artificial subterranean systems, receive several thousand visits per year, mainly from outdoor pursuit centres (Gunn et al., 2000). It is widely accepted that the presence of visitors in subterranean habitats significantly alters the temperature and relative humidity of the environment, and the impact on habitat conditions could be even more dramatic when tourism opportunities are improved by the provision of lighting, drainage of excessive water or temperature regulation, e.g. by forced air (Cigna, 1993).

It is also well known that environmental disturbance, primarily as a result of human activity, can seriously degrade these fragile ecosystems (Jasinska, 1996). Waste disposal, quarrying, changes in agricultural practices, deforestation and groundwater abstraction all pose serious threats to the natural functioning of subterranean ecosystems (Hobbs & Gunn, 1998). In groundwater (Malard, 2001) and cave streams (Simon & Buikema, 1997), changes in the quality and quantity of organic matter entering these low-energy systems have been shown to cause significant alterations of the faunal assemblages, resulting in significant changes to the trophic dynamics of the subterranean (hypogean) food webs (Poulson & Lavoie, 2000).

The subterranean environment in Piedmont and its fauna

There are 2157 wild caves known in the Piedmont region (NW Italy) (data from Regional Speleological Register plus unpublished data), located mainly in the Alps. Only 20 of them are listed, directly or indirectly, in the regional list of Special Conservation Areas (norms LR 47/95, DGR 419-14905, DGR 17-6942). Nearly 200 artificial caves are known in the region, a few of them harbouring rare and peculiar species assemblages, like the above-mentioned military bunker of Vernante (CN) or the underground network of passages of the Chiusa Pesio Abbey (CN) (Bologna & Vigna-Taglianti, 1985).

The biospeleology of Piedmont has been relatively well studied compared to other surface ecosystems, with a huge number of taxa recorded from different cave sites and a high abundance of restricted endemic species. There are many specialized subterranean taxa - obligatory occupants of the subterranean habitats that could not live elsewhere (sensu Giachino & Vailati, 2010) and less specialized subterranean taxa (“troglophilic”) - that can maintain permanent populations in caves, although not restricted to that environment (Chapman, 1993). In the Piedmontese Alps, from Lago Maggiore to the Ligurian Alps, there are around 150 specialized subterranean species - “eucavernicolous” according to Ruffo (1995) - with approximately 100 steno-endemic species, mainly concentrated in the southern part of the Alps on the border between Piedmont and Ligury (Bologna & Vigna-Taglianti, 1985).

A preliminary biospeleological survey carried out in 2007 at the Fontane mine complex by the authors and several collaborators revealed an interesting assemblage of species, characterized by several specialized subterranean organisms including a new subspecies of ground beetle (Coleoptera, Carabidae) (Casale & Giachino, 2008) and an unidentified species of stygobiontic amphipod (Malacostraca, Amphipoda) found in the small rivulets running in the mine tunnels.

In view of the peculiar species assemblage in the abandoned Fontane mine complex, the aims of the present work were (i) to investigate the invertebrate cave fauna of the mine complex and its habitat by a full environmental characterization and comparison to a wild cave in order to identify the main factors influencing the species assemblages, (ii) to assess the impact of tourism activities in one of the tunnels on the invertebrate fauna, with special regard to the most interesting species, and (iii) to assess the conservation value of the terrestrial invertebrate community and associated habitats within the mine complex in order to provide general guidelines aimed at minimizing the negative effects of human intervention.

METHODS

Study area

The Fontane mine complex is located in the municipality of Prali, Germanasca Valley, Cottian Alps, Piedmont (NW Italy) (Figure 1). Geologically, the Germanasca Valley is in the Dora-Maira crystalline massif of the Pennidic domain of the Western Alps.

The precipitation regime of the area is Western prealpine, with a bimodal trend (peaks in spring and autumn, minima in winter and summer). The mean annual precipitation is around 1100 mm. Temperatures follow a unimodal trend, with the peak in August (18°C) and minimum in January (0.1°C). The mean annual temperature is 9.1°C. According to the Thornthwaite classification (Thornthwaite, 1948), the climate at Prali station (a few hundred metres from the Fontane mine complex) is humid, cold microthermic with a mild summer and a low or absent water deficit (Biancotti & Bovo, 1998). Concerning the soil climate, the humidity regime is udic and the soil temperatures range from mesic (8-15°C at 50 cm deep) at lower altitudes to cryic (0-8°C at 50 cm deep) at higher altitudes.

The Fontane mine complex is well known for its important talc seam, consisting of a unique belt crossing the valley in a N-S direction, with total development of 2 Km and thickness ranging from 1 to 10 m (Sandrone et al., 1987). The industrial extraction of talc in the Fontane area is documented since 1780. In modern times, the Fontane mine complex has been considered one of the most important European talc mining sites, in terms of both quantity (40,000 tons per year) and quality (80% pure white talc in lamellar structure) (Cian et al., 1984).

The mine complex is situated on both sides of the Germanasca Valley at an altitude of 1140 to 1520 m. The earliest extraction (1780-1883) was performed on superficial outcrops. From 1883 to the present, mining was carried out underground, with the excavation of more than 20 tunnels. Today, extraction has ceased in most of the tunnels, although one is still active (Rodoretto mine). Two tunnels (Paola and Gianna) were restored for tourism by the Regional Ecomuseum of Germanasca Valley in 1998 (Paola) and 2001 (Gianna). The tunnels are open 12 months a year and are visited on average by 22,000 visitors annually (total visitors: 225,700 since the restoration).

The present study was conducted at four sites located about 2 Km east of the village of Fontane in the same valley: one tunnel restored for tourism purposes (Gianna), two abandoned tunnels (Gianfranco and Santa Barbara) and a wild cave (Chiabrano, Tuna del Diau) (Figure 1).

Gianna tunnel (G) was opened in 1935, closed in 1995 and restored for tourism in 2001. There are less visitors (around 1000 per year) than to Paola tunnel, which accounts for the maximum tourist flow. Despite the sporadic presence of tourists, the tunnel has been massively renovated, with an electrical system for lighting and emergency, a water drainage system (structured in tubes and open rivulets) and a forced-air system of fans and compartmentation doors, also used in case of fire emergencies. The tunnel, opening at 1212 m a.s.l., is a horizontal double-ended corridor extending for over 2000 m with several small lateral branches about 10 m long. Compartmentation fire doors are located at 250 m (door A) and 1200 m (door B) from the entrance. Beyond door B, the tunnel walls have been lined with cement. The rocky ground is covered with gravel, clay and, mostly in the lateral branches, huge quantities of decaying wood.

Gianfranco tunnel (GF) was opened in 1932 and closed in 1972. After its closure, the mine was abandoned and, as far as we know, only a few speleologists have entered the tunnel. It is in an evident state of abandonment, with many damaged sections, collapses of scaffolding, abundant running water, breakdowns of the iron and wood frameworks and unprotected potholes on the sides of the main corridor. The tunnel opens at 1377 m a.s.l. and extends horizontally for nearly 2000 m (total development). After approximately 400 m, the main corridor reaches a lateral branch with an outside exit after 200 m and then continues for nearly 400 m, where a collapse blocks the passage. A few small lateral branches are found on the side of the main corridor. The ground is mainly covered with material originating from the collapses (decaying wood, iron, rocks, stones, gravel, sand, clay and other kinds of debris). Running water is found the whole year on the sides of the main corridor.

Santa Barbara tunnel (B) is located a few hundred metres south of Gianfranco tunnel, at an elevation of 1307 m a.s.l. It was opened in 1910 and closed in 1965. Like Gianfranco tunnel, it is in an evident state of abandonment, with collapses and running water along the main corridor. The entrance is very remote and probably only a few speleologists have visited the mine in the last century. It extends horizontally for nearly 2000 m but after approximately 400 m from the main entrance the passage is blocked by a collapse with water gushing out at different heights, suggesting that the tunnel beyond the collapse is overfilled with water. One small lateral branch (20 m) is located 200 m before the collapse. The ground is covered with gravel, mud and rocks, while decaying wood is rarely found. Running water is found the whole year in rivulets on one side of the main corridor.

The wild cave of Chiabrano “Tuna del Diau” (CH) is located nearly 2 Km east of the mines in the village of Chiabrano (municipality of Perrero). It is a subhorizontal tectonic fracture opening at 1150 m a.s.l. in a larch wood on a west-facing slope, and has a total development of approximately 50 m. It was censused in 2005 by Enrico Lana and Renato Sella (census number 1621 Pi/TO in the Regional Speleological Register). The cave has two narrow openings, one of them not practicable. Litter, stones and rocks cover the ground. No running water is found, but water often drips from the walls.

Sampling design

Fifteen pitfall traps (plastic cups diameter 65 mm, volume 150 ml) were used to sample arthropods in the mine tunnels and in the wild cave. We baited the traps with cheese and filled them with 30 ml 50% ethylene glycol as a killing–preserving solution. The traps were covered with stones. Traps were replaced approximately once a month from January to June 2007. Due to problems of access to the mines from July to mid-August (access roads blocked due to floods and landslides), trapping was suspended for two months. Further sampling was performed in September 2007, for a grand total of 90 samples. The small number of traps per site and the relatively short sampling period were chosen to limit the impact of the study on the local population of specialized subterranean taxa (Casale et al., 1996). On each visit, we were able to verify that the high number of arthropods walking on the soil and walls was never affected by the presence of traps. Five traps (G1-G5) were placed in Gianna tunnel at 260, 620, 700, 730 and 1200 m from the entrance. G5 was situated before the compartmentation fire door. Four traps (GF1-GF4) were placed in Gianfranco tunnel at 260, 480, 700 and 720 m from the main entrance. Five traps (B1-B5) were placed in Santa Barbara tunnel at 30, 40, 120, 240, 280 m from the main entrance. One trap was placed in Chiabrano wild cave (CH) at 10 m from the opening. Figure 1 shows the positions of the traps.

Ten environmental factors that could affect the distribution of arthropods were measured. Data on soil cover referred to % of litter (1), rocks (2), gravel (3), stones (4) and decaying wood (5) within a circle (5 m radius) centred on the trap. We measured the distance from the entrance (6) and from the nearest running water (7) and we estimated the local dampness (8) (4 values ranging from 0 to 3) for each sample. Five measurements of air temperature were taken with a portable digital thermometer at different points around the trap (one measurement on the trap and four at a 5-m distance at 0°, 90°, 180° and 270°). Mean values (9) and standard deviations (10) of the five measurements for each monthly sample were used in the statistical analysis (standard deviation was used to obtain a measurement of environmental thermic variability).

Trapped invertebrates were sorted and identified to the species level (whenever possible) using updated standard keys or specialist works. The nomenclature follows the check-list of Italian species (Stoch, 2003). Due to the lack of a recent national updating, the spider nomenclature refers to Platnick’s world catalogue (Platnick, 2010). A number of invertebrates (Oribatida, Julida Blaniulidae and Collembola Onychiuridae) could not be identified to the species level. These individuals were included as morpho-species, indicated as the genus followed by sp.

Data analysis

We used two-way ANOVA with LSD post-hoc test to test differences among sites and in relation to the sampling period of the mean frequencies of activity densities (N: $\sum_{i=1}^{n} t_i$ where t_i is the number of active traps per sampling day and n the total number of sampling days), species richness (number of taxa collected, S) and diversity (Shannon index, SH) of arthropods in each trap. The null hypothesis was that the community parameters were equivalent in all groups and that the period did not differentially affect them. Normal distribution of the data was achieved by log(x + 1) transformation (Sokal & Rohlf, 1995). A level of significance of alpha = 0.05 was used for all analyses.

The initial system of environmental variables was reduced by application of the PCA method (Principal Components Analysis; Gaunch, 1984), which facilitated examination of the data structure and dominant modes of intercorrelation amongst environmental variables. PCA was used to identify the variables accounting for the major sources of variation within the dataset, thus minimizing redundancy. A cross-products matrix contained correlation coefficients among environmental variables. The <environmental variable x sample data> matrix contained 9 variables: mean values and standard deviations of temperature, percentages of decaying wood, stones, gravel and litter, distance from running water, distance from entrance and local dampness. Axes with broken-stick eigenvalues less than the actual eigenvalues for that axis were considered for interpretation (Jackson, 1993) and used to examine the relationships among species assemblages using CCA (Canonical Correspondence Analysis; Ter Braak, 1986). Principal components were included in CCA to reduce the noise introduced by using all the original variables (Mc Cune, 1997). An ordination diagram was produced by detecting patterns of variation in community composition that could best be explained by the environmental variables. The position of a certain species in the resulting plot indicates the characteristics of its ecological optimum (Ter Braak, 1986). A <species x sample data> matrix was produced using log(x+1) transformation to stabilize variances. The species included in the analysis made up 95% of the total sample (Lesica & Cooper, 1998). The resulting matrix was composed of 64 observations and 7 species. The environmental matrix consisted of the corresponding 64 observations and 3 variables obtained by PCA. The site scores were centred with unit variance (biplot scaling). A Monte Carlo test of significance was performed to test the null hypothesis of no linear relationship between matrices (1000 randomizations).

RESULTS

In total, 2550 cave-dwelling arthropods, belonging to 29 taxa, were collected in the pitfall traps. The total number of taxa rose to 38 when the results of direct hand sampling during the surveys were included (Table 1). The most abundant species (31.29%) was *Doderotrechus crissolensis* (Dodero, 1924) (Coleoptera, Carabidae), found exclusively in Gianfranco tunnel, followed by an unidentified blaniulid species (Diplopoda, Julida) (29.92%), *Doderotrechus ghilianii isaiai* (Casale & Giachino, 2008) (Coleoptera, Carabidae) (21.84%), *Alpioniscus* sp. (Isopoda, Trichoniscidae) (7.60%), an unidentified onychiurid species (Hexapoda, Collembola) (2.98%), *Limonia nubeculosa* (Diptera, Limoniidae) (1.50%) and unidentified oribatid mites (Oribatida) (1.50%). Several other hypogean and troglophilic species were sampled in the tunnels by direct hand sampling, such as *Niphargus* sp. (Amphipoda, Niphargidae), *Dolichopoda ligustiga* (Orthoptera, Rhaphidophoridae) and *Amilenus auranticus* (Opiliones, Sclerosomatidae) (Table 1).

Despite the proximity among sampling sites, the species composition was rather different (Figure 2). The four study sites showed substantial differences in coleopterans, especially in the most specialized subterranean species. For example, *Doderotrechus ghilianii isaiai* was found in Gianna and Gianfranco tunnels, while it was absent in Santa Barbara tunnel and in Chiabrano wild cave. The congeneric *D. crissolensis* was only found in Gianfranco tunnel. Chiabrano wild cave was characterized by a substantially different faunal assemblage, especially concerning subterranean specialized species of coleopterans and spiders. Both *Doderotrechus* species were absent, while the specialized subterranean *Dellabeffaella olmii* (Coleoptera, Cholevidae) and the troglophilic *Troglohyphantes vignai* (Araneae, Linyphiidae) were found exclusively there. An unidentified blaniulid species (Diplopoda, Julida), *Limonia nubeculosa* (Diptera, Limoniidae), an unidentified onychiurid species (Collembola) and *Porrhomma convexum* (Araneae, Linyphiidae) were the only taxa found at all four study sites.

Gianna and Gianfranco tunnels showed similar relatively high mean values of activity density (N) (1.00 and 1.33 invertebrates/trap/day respectively) compared to Santa Barbara tunnel and Chiabrano wild cave (0.04 and 0.07 invertebrates/trap/day respectively). The highest number of taxa was recorded in Gianna tunnel (18) (see Table 1). Chiabrano wild cave had the highest mean value of species richness (4.75 species/trap), while Santa Barbara had the lowest (1.24 species/trap).

The total abundance, species richness and diversity of arthropods were significantly different among the four sites (Table 2). The LSD post-hoc test revealed that Santa Barbara tunnel had lower values of activity density, species richness and Shannon diversity index than Gianna and Gianfranco tunnels. Species richness was significantly higher in Chiabrano wild cave than in Santa Barbara and Gianfranco tunnels, while the Shannon diversity index was significantly lower in Santa Barbara tunnel than in the other two tunnels (Gianna and Gianfranco) (Table 3 and Figure 3). The sampling period differences and interactions were not significant, suggesting that the sampling period had no effect and the effect of habitat did not depend on the effect of time; hence, there was neither synergism nor interference between habitat and time factors. Therefore, we considered each sample as independent in further analyses.

The mean temperatures in Santa Barbara (9.36°C) and Gianfranco (8.58°C) tunnels were comparable among the trap positions, with relatively low variability (min 8.5°C and 7.00°C and max 9.8°C and 9.5°C, respectively). Indeed, the temperatures at Santa Barbara were particularly stable. In agreement with Smithson (1991), the mean temperature in the inner part of the tunnels closely corresponded to the mean annual temperature outside the tunnels. In contrast, the temperatures in Chiabrano wild cave were lower and more unstable (mean: 7.03°C, min: 4°C and max: 9.8°C). Temperatures in Gianna tunnel were particularly unstable, ranging from 7.00°C (recorded at G5, the only trap placed before the compartmentation fire door) to 15.40°C (recorded at G1, at the very core of the tunnel) (Figure 4). Soil cover differed mainly in terms of percentages of decaying wood and, conversely, gravel, ranging from 0 to 90%. Litter was only found in Chiabrano wild cave. Traps placed after the compartmentation door in Gianna tunnel were generally drier and more distant from running water than the others. Running water was absent in Chiabrano wild cave, but the environment was generally damp.

The first three principal components (PC1, PC2 and PC3) accounted for 80.96% of the total variation in the environmental matrix**,** with broken-stick eigenvalues less than the actual eigenvalues for that axis. The percentage of decaying wood and distance from the entrance provided the major negative loadings on PC1 (39.68%), suggesting a disturbance gradient from the natural non-eutrophic sites closer to the exterior to the human-induced eutrophic habitat conditions found in the inner parts of the tunnels. Standard deviations of temperatures showed the highest positive loadings on PC2 (30.27%), suggesting an environmental stability gradient from the unstable habitat of Chiabrano wild cave to the stable inner part of Gianfranco and Santa Barbara tunnels, characterized by strong thermic stability. Distance from running water also showed negative loadings on PC2, with Gianfranco and Santa Barbara tunnels characterized by the presence of running water for the whole year. Mean temperature loaded positively on PC3 (11.01%), suggesting a thermic gradient from cold sites to warmer ones. Summary statistics for PCA are listed in Table 4. The relative position of the pitfall traps and centroids (i.e. the average pitfall traps in ordination space) in the biplot determined by the first two principal components (Figure 5) showed that the Chiabrano wild cave trap site clearly stood apart in terms of environmental variables: it was typified as non-eutrophic, unstable, close to the exterior and rich in litter. For the tunnels, we identified an induced eutrophy + environmental instability gradient ranging from Gianna (eutrophic, unstable) to Gianfranco (eutrophic, stable) to Santa Barbara (non-eutrophic, stable).

The results of the CCA of arthropod activity density in relation to the environmental variables (-eutrophy and -distance from entrance, PC1; +environmental instability and +percentage of litter, PC2; +mean temperature, PC3) are shown in Table 5. Axes 1 and 2 evaluated with a Monte Carlo test with 1000 permutations were significant ($p<0.05$). Figure 6 shows the ordination of species and sites based on LC scores (linear combination of environmental variables). Intraset correlations (sensu Ter Braak, 1986) of environmental variables indicated that environmental instability (PC2) and temperature (PC3) were the main environmental variables influencing the ordination (respectively -0.899 and -0.860). The third variable (PC1, -eutrophy and -distance from entrance) showed a lower positive correlation with the first axis of ordination (0.392, see summary statistics in Table 5). The approximate ranking of the centres of species distributions suggested that the more specialized arthropods (*Doderotrechus crissolensis* and *Doderotrechus ghilianii isaiai*, namely Carabidae), together with mites (Oribatida sp.) and springtails (Onychiuridae sp.), were negatively affected by environmental instability, while *Alpioniscus* sp. (Malacostraca, Isopoda), *Limonia nubeculosa* (Hexapoda, Diptera) and Blaniulidae sp. (Diplopoda) were associated with higher levels of instability. *D. crissolensis* seemed to be the species most sensitive to thermic instability, a factor that could explain its exclusion from Gianna tunnel. There was a similar trend for carabids and blaniulids concerning the temperature gradient, carabids preferring cold temperatures and blaniulids showing a preference for warmer (= more eutrophic) conditions. Cold sites were also preferred by *Alpioniscus* sp. (Isopoda), while Onychiurida sp. and Oribatida sp. showed a preference for warmer conditions. Eutrophic and inner habitats were dominated by detritivorous taxa, such as *Alpioniscus* sp. (Isopoda) and Blaniulidae sp. (Diplopoda), whereas predators, namely carabids (*Doderotrechus* spp.), characterized intermediate eutrophic conditions.

DISCUSSION

In view of their environmental peculiarities and the presence of obligatory subterranean species assemblages, hypogean habitats are of considerable scientific interest (Tercafs, 1988). Indeed, European Habitat Directive 43/92 lists “Caves not open to the public” as a “natural habitat type of community interest whose conservation requires the designation of special areas of conservation” (Natura 2000 code: 8310). The definition is interpreted as referring to natural caves, which are not routinely exploited for tourism and which host specialist or endemic subterranean species or support important populations of Annex II species. As stated in the introduction, artificial subterranean habitats sometimes show the same peculiarities as natural subterranean ecosystems, hosting important populations of subterranean short-range species, and they should be considered as equivalent. Despite their potential conservation importance, guidelines or suggestions for the management of subterranean ecosystems (artificial or natural) have rarely been proposed (see for example Watson et al., 1997); moreover, as far as we know, cave-dwelling invertebrates have never been part of conservation actions despite their general sensitivity to environmental stress. As pointed out in the literature (Balletto & Casale, 1991; Mc Geoch, 1998), invertebrates are small, diversified and sensitive to environmental variability, and thus are good indicators of ecosystem diversity and human disturbance. For example, carabids and spiders have been identified as sensitive indicator taxa in natural and disturbed systems and may show clear responses to environmental disturbances (Pearce & Venier, 2006). Both taxa were found in the present study and, especially for carabids, there seemed to be a negative influence of human-induced alteration of the local microclimate (increased thermic instability and mean temperatures) on sensitive cave-dwelling species.

Our study highlights the very rapid colonization of an artificial subterranean system by specialized steno-endemic fauna inhabiting the endogean fissures system of non-carbonatic rocks. As stated in the description of the study sites, these tunnels were opened nearly 100 years ago and closed less than 45 years ago: Gianna tunnel was opened in 1935 and closed in 1995, Santa Barbara tunnel was opened in 1910 and closed in 1965 and Gianfranco tunnel was opened in 1932 and closed in 1972. It should be emphasized that this is the first case study, at least in Italy, where the precise beginning of the re-colonization process by the subterranean fauna is known.

Despite their substantial differences (see Results section), the species assemblages found in the tunnels of the Fontane mine complex and in Chiabrano wild cave are characterized by several interesting steno-endemic subterranean elements, indicating the high potential of the whole area for conservation purposes. Particular interest was aroused by the syntopic co-existence of two species of *Doderotrechus* (Coleoptera, Carabidae), never recorded before for this genus. Despite a few cases of sympatry reported in the literature in which a distinct selection of the habitat was shown, their complete syntopic occurrence in Gianfranco tunnel, both being represented by very abundant populations (Figure 2), is extraordinary (Casale & Giachino, 2008). In this sense, these environments provide a unique habitat, with unexpectedly high ecological value. The presence of several other vulnerable specialized subterranean taxa (i.e. *Niphargus* sp., *Doderotrechus ghilianii isaiai*) in both Gianna and Gianfranco tunnels clearly shows the importance of these sites for subterranean fauna. Despite the low activity density recorded, the wild cave of Chiabrano differs significantly from the artificial subterranean systems considered in the analysis. The differences concern both faunistic and structural aspects but they are not clearly interpretable when we consider the more specialized subterranean elements like *Dellabeffaella olmii* and *Troglohyphantes vignai,* which were found exclusively in the wild cave. Another interesting point is the absence of any species of *Doderotrechus* in Santa Barbara tunnel, while they were particularly abundant in Gianna (*D. ghilianii isaiai*) and Gianfranco tunnels. The temperature data highlight the extraordinary thermic stability of Santa Barbara tunnel, which could be explained by poor air circulation (Badino, 1995). In fact, the tunnel is apparently overfilled with water beyond the collapse that blocks the passage (see section describing the study sites). This may result in isolation of the tunnel from the fissure system of the main rock (sensu Giachino & Vailati, 2010) which could have prevented colonization by the subterranean fauna. The absence of *D. crissolensis* from

Gianna tunnel is probably related to its higher thermic instability, indicating the particular ecological requirements of this specialized species.

As mentioned by Gunn et al. (2000) for aquatic subterranean habitats, it is relatively difficult to fully outline the conservation value of subterranean invertebrate communities and habitats, mainly because they are so poorly understood. This work aims to improve the knowledge of the main factors affecting and shaping subterranean invertebrate assemblages and to provide some suggestions concerning the management of the artificial subterranean habitats of the Fontane mine complex. Special attention is given to the impact of tunnel restoration for tourism on the most specialized subterranean species.

The potential impacts on cave ecosystems can be broadly divided into two groups: those external to the cave and those internal to the cave (Gunn et al., 2000). External impacts usually pose the most serious problems, potentially altering sediment loads, subsurface hydrology and both clastic (sediment) and chemical water quality (Watson et al., 1997). Internal impacts on subterranean ecosystems come from intensive uncontrolled tourism and from recreational caving; they could result from inappropriate lighting and the presence of visitors, causing changes in the relative humidity, air temperature and carbon dioxide concentration, as well as alterations of the optimal living conditions of the troglobitic and troglophilic fauna. All these effects would bring about a gradual decline of the environmental quality, favouring the degradation of speleothems (Baker & Genty, 1998; Sánchez-Moral et al., 1999), cave art or troglobitic and troglophilic biodiversity (Mann et al., 2002; Richter et al., 1993). In addition, the presence of lighting often leads to temperature increases (Cigna, 1993) and to the development of floral communities in illuminated areas (Stoch, 2002), while artificial ventilation may cause changes in temperature and humidity, and thus evaporation from cave habitats, including standing water pools (Gunn et al., 2000). Our study suggests that another cause of temperature increase was the installation of compartmentation fire doors, causing thermic isolation of certain parts of the tunnels in which large amounts of wood were decomposing.

The artificial subterranean system of the Fontane mine complex is an excellent site for research on subterranean ecology, as it presents an interesting gradient of trophic conditions and human intervention together with an extremely interesting faunal assemblage. The results of our comparison of the oligotrophic habitat in Santa Barbara tunnel and the eutrophic one in Gianfranco tunnel demonstrate that anthropogenic eutrophic conditions can improve the quality of subterranean habitats. This is particularly evident from the higher values of activity density in the eutrophic tunnels (Gianfranco and Gianna) than in the oligotrophic ones (Santa Barbara and Chiabrano wild cave, see LSD post-hoc test results, Table 3). On the other hand, a massive restoration for tourism could seriously jeopardize the survival of such peculiar coenoses. It is evident from our results (Figure 6) that the increase of mean temperature and its variability induced by wood decomposition in tunnels partially isolated by forced air and compartmentation doors could have a significant negative affect on the local populations of cave-dwelling arthropods, especially the most sensitive and specialized ones like the two syntopic species of specialized hypogean ground beetles. In other words, the human-induced eutrophy found at Gianfranco tunnel has caused a general increase of the carrying capacity of the local ecosystem, favouring the establishment of a noteworthy biocoenosis of specialized subterranean species; however, this biocoenosis could be seriously jeopardized by further interventions leading to a modification of the local microclimate. This is particularly evident from the CCA results in which environmental stability stands out as the major factor influencing species ordination. The ecological optimum for the most sensitive and ecologically important taxa is determined primarily by low thermic variability (high environmental stability), relatively cold temperatures and intermediate conditions of eutrophy. This is particularly true for predator taxa like *Doderotrechus* spp. (Coleoptera, Carabidae), indicating the role of these environmental factors on the complexity of the cave food web. In contrast, valuable steno-endemic species are missing in conditions of thermic instability, higher temperatures and higher eutrophy, typical of the inner parts of Gianna tunnel, subjected to higher human disturbance. The greater environmental instability in Gianna tunnel would also explain the absence of *D. crissolensis*, which can be regarded as the most sensitive species. The assemblages found in this environment are typified by detritivorous taxa, such as diplopods, and by less specialized taxa (subtroglophylous), such as *Limonia nubeculosa* (Diptera).

Our results did not demonstrate any direct effect of visitor frequentation on the invertebrate assemblages. Despite what would be expected and can be observed empirically for other specialized arthropods in wild caves or for bats or any other cave-overwintering vertebrates (Mann et al., 2002; Martin et al., 2006), we cannot suggest any particular period that should be banned to visitors because we did not record any significant temporal changes in the arthropod communities. The ecological peculiarities of the tunnels, in terms of eutrophy and temperature, could explain the absence of temporal effects on the arthropod assemblage.

On the basis of our results, we strongly advise against thermic isolation of any part of mine tunnels by compartmentation fire doors, as this is the main factor affecting the most sensitive and conservationally important species. In the case of the Fontane mine complex, we recommend the closure of compartmentation fire doors only for emergency purposes, so as to avoid excessive heating and to limit thermic variability.

This study has shown that terrestrial cave-dwelling invertebrate communities in abandoned mines are of greater scientific interest than previously supposed, that they are exposed to several risks related to a variety of human activities, and that they are worthy of conservation. For a better understanding of the role of human influence on environmental factors that might affect the invertebrate communities, it would be advisable to conduct similar studies in other artificial and natural subterranean habitats. The direct impact of visitors is another interesting topic that could be investigated in restored tunnels with high tourist flows. For example, Paola tunnel of the Fontane mine complex would be an interesting study area in which to explore this topic; in fact, several preliminary surveys have failed to record the presence of any specialized cave arthropods. Nevertheless, as recommended by Gunn et al. (2000), special care should be taken when sampling subterranean environments, and unnecessary disturbance of habitats and fauna should be avoided whenever possible.

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Figure captions

Figure 1 – Study area. G: Gianna tunnel, GF: Gianfranco tunnel, B: Santa Barbara tunnel, CH: Chiabrano wild cave (same scale as main figure). The map shows the entrances of the tunnels and the entrance of the cave (black dots and codes). Positions of traps in the tunnels (tunnel code and number of trap), compartmentation fire doors (Fire door A and B) and collapses (triple bars across the tunnels found in B and GF) are also illustrated.

Figure 2 – Percentage of the dominant species according to sampling sites (G: Gianna tunnel; GF: Gianfranco tunnel, B: Santa Barbara tunnel, CH: Chiabrano wild cave).

Figure 3 – Differences in diversity (SH), activity density (N) and species richness (S) of collected arthropods among sites (G: Gianna tunnel; GF: Gianfranco tunnel; B: Santa Barbara tunnel; CH: Chiabrano wild cave). Values are means and error bars are ± standard errors. Different letters placed above bars denote significant differences ($p>0.05$) among sites.

Figure 4 – Mean air temperature, standard error (bars), maximum (black dots) and minimum (white dots) recorded at the four study sites. Values are based on 150 measurements for Gianna (G) and Santa Barbara (B) tunnels, 120 for Gianfranco tunnel (GF) and 30 for Chiabrano wild cave (CH). Measurements were performed monthly from January to July and in September 2007 (see text for further details). Dotted line indicates local mean annual temperature (9.1°C).

Figure 5: Biplot of principal components analysis (PC1 vs. PC2). Distribution of pitfall traps and relative centroids are shown. Dashed ellipsoids group, from left to right, the Gianna, Gianfranco, Santa Barbara and Chiabrano wild cave pitfall traps. PC1 and PC2 suggest gradients of human-induced eutrophy and environmental instability, respectively.

Figure 6 - Ordination of species and sites in environmental space as defined by CCA, using LC scores. Triangles are sites , + symbols are species. Legend refers to sampling sites.

TABLES

Table 1 - List of taxa collected at the four study sites by means of pitfall traps and hand collection (G: Gianna tunnel; GF: Gianfranco tunnel; B: Santa Barbara tunnel, CH: Chiabrano wild cave). Spider nomenclature is updated to Platnick's world catalogue (2010), for other taxa we refer to Stoch (2003).

Class	**Order**	**Family**	**Species**	**Occurrence**
Arachnida	Pseudoscorpionida	Neobisiidae	*Roncus* sp.	G
	Opiliones	Sclerosomatidae	*Amilenus aurantiacus* (Simon, 1881)	
		Sclerosomatinae	*Astrobunus bernardinus* Simon, 1879	
	Araneae	Linyphiidae	*Porrhomma convexum* (Westring, 1851)	B CH G GF
			Palliduphantes pallidus (O. P.-Cambridge, 1871)	B GF
			Troglohyphantes vignai Brignoli, 1971	CH
		Pimoidae	*Pimoa rupicola* (Simon, 1884)	B CH G GF
		Agelenidae	*Malthonica silvestris* (L. Koch, 1872)	B CH G GF
		Tetragnathidae	*Metellina merianae* (Scopoli, 1763)	B CH G GF
		Pholcidae	*Pholcus phalangioides* (Fuesslin, 1775)	G
			Pholcus sp. (unidentified remains)	G
			Psilochorus simoni (Berland, 1911)	G
	Oribatida		Oribatida sp.	B G GF
Malacostraca	Isopoda	Trichoniscidae	*Alpioniscus* sp.	G
	Amphipoda	Niphargidae	*Niphargus* sp.	G GF
Chilopoda	Lithobiida	Lithobiidae	*Lithobius forficatus* (Linnaeus, 1758)	G
			Lithobius tricuspis Meinert, 1872	CH
	Scolopendromorpha	Cryptopidae	*Cryptops hortensis* (Donovan, 1810)	G
Diplopoda	Julida	Blaniulidae	Blaniulidae sp.	B CH G GF
Hexapoda	Diptera	Limoniidae	*Limonia nubeculosa* Meigen, 1804	CH G GF
	Orthoptera	Rhaphidophoridae	*Dolichopoda ligustica* Baccetti & Capra, 1958	CH G GF
	Coleoptera	Cholevidae	*Dellabeffaella olmii* Casale, 1980	CH
			Catops subfuscus Kellner, 1846	CH
		Carabidae	*Doderotrechus ghilianii isaiai* Casale & Giachino, 2008	G GF
			Doderotrechus crissolensis (Dodero, 1924)	GF
			Platynidius complanatus (Dejean, 1828)	CH

			Pterostichus truncatus (De Jean, 1828)			G	
			Pterostichus vagepunctatus Heer, 1838				GF
			Pterostichus sp. (unidentified remains)			G	
			Sphodropsis ghilianii (Schaum, 1858)		CH		
			Trechus fairmairei Pandellé, 1867			G	
		Staphylinidae	*Quedius mesomelinus silensis* Fiori, 1894			G	
			Aloconota sulcifrons (Stephens, 1832)			G	
			Atheta (Philhygra) hygrotopora (Kraatz, 1856)				GF
	Collembola	Onychiuridae	Onychiuridae sp.	B	CH	G	GF
Gastropoda	Stylommatophora	Zonitidae	*Oxychilus draparnaudi* (Beck, 1837)		CH	G	
		Discidae	*Discus rotundatus* (O.F. Müller, 1774)			G	

Table 2 -Summary statistics for ANOVA (N: activity density; S: species richness; H: Shannon index of diversity; SS: sum of squares, df: degrees of freedom; MS: mean square).

	SS	df	MS	F	P value
Dep. Var: N					
Groups	1.615	3	0.490	7.190	0.004**
Period	1.470	5	0.042	0.614	0.691
Groups x Period	0.210	11	0.068	0.903	0.543
Error	4.416	59	0.075		
Dep. S					
Groups	74.762	3	24.921	7.409	0.004**
Period	5.941	5	1.188	0.356	0.870
Groups x Period	37.599	11	3.418	1.259	0.271
Error	37.599	59	2.715		
Dep. Var: H					
Groups	0.401	3	0.134	9.330	0.000**
Period	0.044	5	0.009	0.614	0.691
Groups x Period	0.157	11	0.014	0.940	0.510
Error	0.894	59	0.015		

Table 3 - Summary statistics for multiple comparisons (LSD post-hoc test) for activity density (N), species richness (S), diversity (SH). G: Gianna tunnel; GF: Gianfranco tunnel; B: Santa Barbara tunnel, CH: Chiabrano wild cave. MD= Mean Difference, SE= Standard Error.

Parameter	(I) group	(J) group	MD (I-J)	SE	Sig.
N					
	G	B	0.254 (*)	0.074	0.001
		CH	0.226	0.146	0.125
		GF	-0.046	0.079	0.560
	GF	B	0.300 (*)	0.082	0.001
		CH	0.273	0.150	0.074
	B	CH	-0.028	0.147	0.852
S					
	G	B	1.893 (*)	0.446	0.000
		CH	-1.617	0.877	0.070
		GF	0.433	0.476	0.366
	GF	B	1.460 (*)	0.494	0.005
		CH	-2.050 (*)	0.903	0.027
	B	CH	-3.510 (*)	0.887	0.000
SH					
	G	B	0.153 (*)	0.033	0.000
		CH	0.081	0.066	0.221
		GF	0.011	0.036	0.761
	GF	B	0.142 (*)	0.037	0.000
		CH	0.070	0.067	0.302
	B	CH	-0.072	0.066	0.279

Table 4 – PCA summary statistics. Variance extracted (first three axes) and factor loadings of the first three eigenvectors. Bold values refer to highest loadings: PC1: percentage of decaying wood and distance from entrance; PC2: standard deviations of temperature and distance from running water; PC3: mean temperature.

Variance extracted, first 3 axes.

AXIS	Eigenvalue	% of Variance	Cum.% of Var.	Broken-stick Eigenvalue
1	3.57	39.68	39.68	2.83
2	2.72	30.27	69.95	1.83
3	1.01	11.01	80.96	0.99

First 3 eigenvectors, factor loadings

Eigenvector	Axis 1	Axis 2	Axis 3
Environmental variables			
Mean temperature	-0.57	-0.06	**0.73**
Standard deviations of temperature	-0.46	**0.84**	-0.17
Percentage of decaying wood	**-0.93**	0.29	-0.34
Percentage of stones	0.34	**0.70**	0.32
Percentage of gravel	0.56	-0.67	0.16
Percentage of litter	0.65	0.68	-0.05
Distance from running water	0.39	**0.80**	0.04
Distance from entrance	**-0.88**	-0.16	0.14
Local dampness	0.64	-0.34	-0.39

Table 5 – CCA axis summary statistics.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.371	0.105	0.008
Variance in species data:			
% explained	16.8	4.7	0.4
Pearson Correlation, Spp-Envt	0.704	0.573	0.175
Intraset correlation for environmental variables			
1 PC1 (- eutrophy)	0.392	-0.362	0.846
2 PC2 (instability)	-0.899	-0.066	0.432
3 PC3 (mean temperature)	-0.454	-0.860	-0.234

Weighted correlations among variables in environmental matrix
(weighted by row totals in species matrix)

	PC1	PC2	PC3
1 PC1 (induced eutrophy)	1.000		
2 PC2 (instability)	0.037	1.000	
3 PC3 (mean temperature)	-0.061	0.364	1.000