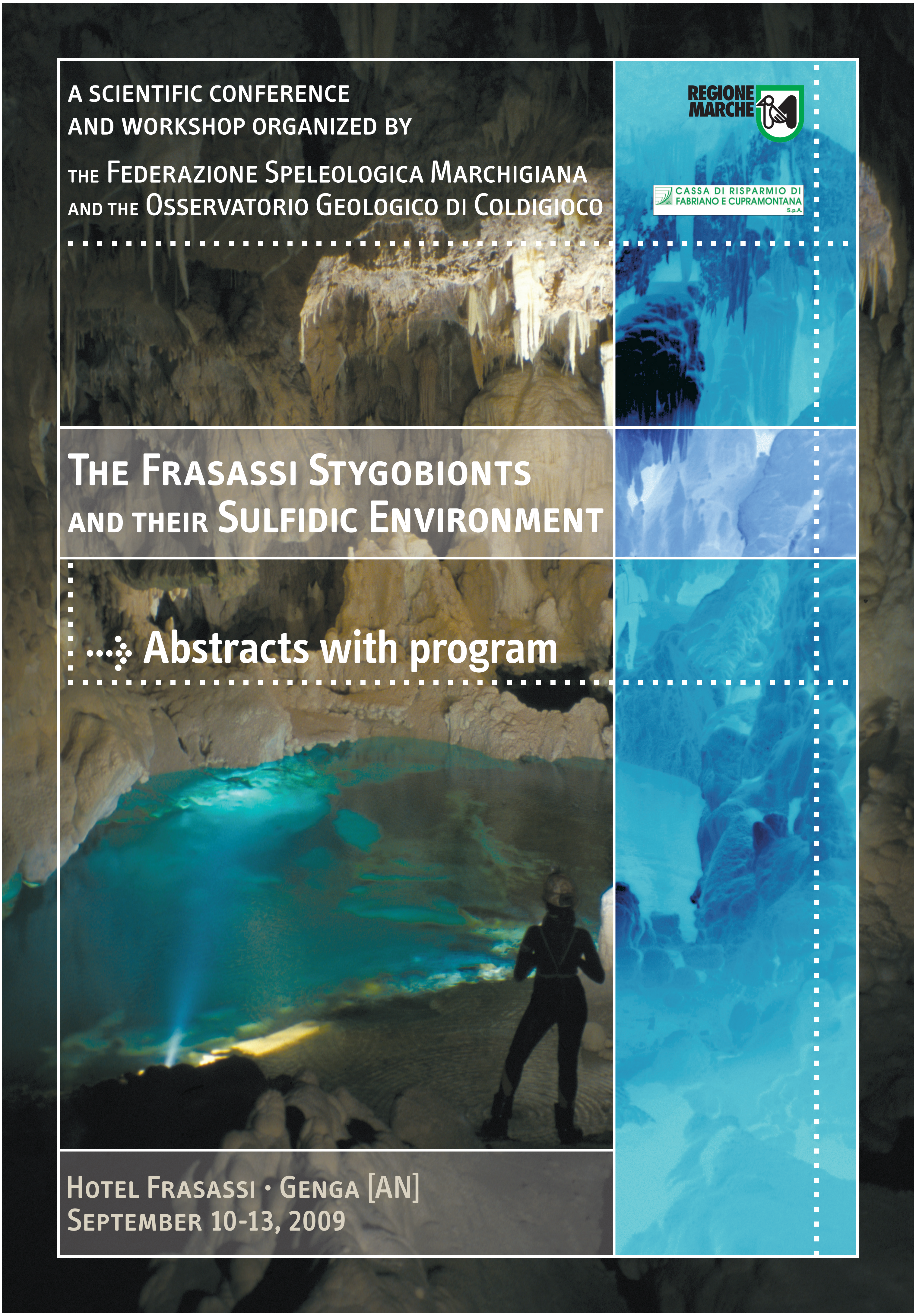

A SCIENTIFIC CONFERENCE
AND WORKSHOP ORGANIZED BY

THE FEDERAZIONE SPELEOLOGICA MARCHIGIANA
AND THE OSSERVATORIO GEOLOGICO DI COLDIGIOCO

REGIONE MARCHE

CASSA DI RISPARMIO DI
FABRIANO E CUPRAMONTANA
S.p.A.

THE FRASASSI STYGOBIONTS AND THEIR SULFIDIC ENVIRONMENT

Abstracts with program

HOTEL FRASASSI · GENGA [AN]
SEPTEMBER 10-13, 2009

The Frasassi Stygobionts and their Sulfidic Environment

a scientific conference and workshop organized by
the Federazione Speleologica Marchigiana, and
the Osservatorio Geologico di Coldigioco

Hotel Frasassi - September 10-13, 2009

PROGRAM

Thursday 10

8:00 Breakfast

9:00 Welcome by the local authorities

9:20 Introduction: the FSM-OGC research program on the aquatic fauna of the Frasassi cave complex
A. Montanari

9:30 Geologic setting of the Frasassi cave complex
A. Montanari

10:15 The hypogene caves of the Apennines, with special regard to the Frasassi karst
S. Galdenzi

10:45 Coffee break

11:05 Environments and technical aspects of the Frasassi cave complex
S. Mariani, M. Mainiero, S. Cerioni etc.

11:50 Stygobionts of the Frasassi cave complex: a review
A. Montanari

13:00 Lunch break

Afternoon excursion

15:00 Geological hike through the Frasassi stratigraphic succession
16:00 Visit the geo-speleologic museum at San Vittore
16:30 Visit the Frasassi Sulfidic Spring
17:30 Visit the Grotta Grande del Vento (tourist part)
19:00 Return to the Hotel Frasassi

20:00 Dinner

Friday 11

8:00	Breakfast
9:00	Sulfur isotope geochemistry relating to the Frasassi cave system *T. Maruoka and S. Galdenzi*
9:30	Biological controls on detailed sulfur cycling and the formation of the Frasassi caves *G. Druschel, D. Eastman, and J. L. Macalady*
10:00	S isotope investigation of sulfur cycling in the Frassassi cave system: A case study of chemotrophic sulfide oxidation *A. Zerkle, J. L. Macalady, and J. Farquhar*
10:30	Coffee break
11:00	Metabolic activity of a cyanobacterial mat in a sulfidic spring emerging from the Frasassi cave system, Italy *S. Häusler, J. Klatt, and L. Polerecky*
11:30	Microbial food webs in Movile Cave, Romania *C. Murrell*
12:00	Microbial life along environmental gradients in karst habitats *J. Mulec, A. S. Engel, J. Walochnik, A. Oarga, G. Kosi, A. Krivograd Klemenčič*
12:00	Sulfur oxidizing extremophiles from the caves of Acquasanta Terme, Italy *D. S. Jones, D. Tobler, I. Shaperdoth, S. Galdenzi , M. Mainiero, and J. L. Macalady*
13:00	Lunch break
15:00	Frasassi's microbial sulfur world: Progress and prospects *J. L. Macalady, D. S. Jones, S. Dattagupta, I. Schaperdoth, H. L. Albrecht, K. S. Dawson, D. Tobler, S. Galdenzi, S. Cerioni, and S. Mariani*
15:30	Metagenomic, phylogenetic, and culture-based analysis of extremely acidic sulfidic cave snottites *D. S. Jones, I. Shaperdoth, J. H. Patel, and J. L. Macalady*
16:00	Enigmatic archaea from the dark, anoxic terrestrial subsurface *J. L Macalady, D. S. Jones, R. McCauley, I. Shaperdorth, D. Bloom, and S. Mariani*
16:30	Coffee break
17:00	Cave Biology and the Replicated Evolutionary Experiment *R. Borowsky*

17:30 The protozoan ciliates of the Frasassi caves: a potential source of bioactive molecules for biotechnological applications
F. Buonanno, Chandramohan, B. Di Pretoro, A. La Terza, C. Ortenzi, and Santosh Kumar

18:00 A possibly opportunistic clitellate annelid belonging to the genus *Potamothrix* (family Naididae) in the microbial ecosystems of the Frasassi caves (Italy)
C. Èrseus

18:30 Mollusks of the karstic complex of Frasassi
M. Bodon, S. Cianfanelli, and A. Montanari

20:00 Dinner

Saturday 12

8:00 Breakfast

9:00 Reconnaissance of ostracod assemblages in the Frasassi cave system, Sentino River, and adjacent sulfidic spring
D. Peterson, A. Montanari, and S. Mariani

9:30 What lies beneath: diversity, ecology and evolution of stygobiotic copepods (Crustacea, Copepoda)
D. Galassi, B. Fiasca, E. Insom, and A. Montanari

10:00 The subterranean genus *Niphargus* Schiodte, 1847 (Amphipoda, fam. Niphargidae), taxonomy, origin, and relationships between Italian and Balkan members of this genus
G. Karaman

10:30 Coffee break

11:00 Subterranean assemblages of the genus *Niphargus*: the role of functional morphology
C. Fišer and S. Prevorčnik

11:30 Observations on *Niphargus* in the Frasassi Cave System
B. Borowsky

12:00 The *Niphargus* symbiosis from the Frasassi Cave ecosystem: a case of multiple partners?
S. Dattagupta

12:30 Ecology and diversity of an amphipod – bacterial ectosymbiosis from the Frasassi cave complex in Central Italy
J. Bauermeister

13:00 Lunch break

Afternoon

15:00	Discussion on future projects and the proceeding volume
20:00	Dinner

Sunday 13

8:00	Breakfast
9:00	Excursions guided by the FSM speleologists to observe and sample different lakes within the Frasassi caves (Bugianardo, Grotta Solfurea, Lago Verde, Pozzo dei Cristalli, Lago Claudia).
13:00	Lunch break
15:00	Visit the Geomicrobiolab and the mesocosms at the Geological Observatory of Coldigioco, with local wine, cheese, and traditional food tasting
20:00	Last supper at Hotel Frasassi or Festa della Polenta in Apiro (weather permitting)

ABSTRACTS

Ecology and diversity of an amphipod – bacterial ectosymbiosis from the Frasassi cave complex in Central Italy

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A symbiotic relationship between the troglobitic *Niphargus* amphipod and chemoautotrophic bacteria has been recently discovered in the hypogean karst complex of Frasassi (Genga, Italy). Hitherto, the diversity of this chemosynthetic association has been underestimated. *Niphargus ictus* has so far been assumed to be the only *Niphargus* species in sulfidic streams and lakes of the cave system. Molecular and morphological analyses, however, revealed the presence of four different cave-dwelling *Niphargus* populations. According to behavioral observations, some species are active swimmers, populating calm and deep waters, whereas others prefer to crawl on sediment and rocks in flowing streams or near cave springs. Molecular fingerprinting techniques were employed on *Niphargus* samples from different cave sites to investigate the diversity and eventual distribution patterns of their associated bacterial epibionts. Astonishingly, preliminary results from automated ribosomal intergenic spacer analyses (ARISA) suggest that the composition of the epibiotic communities correlates rather with the host species than with environmental conditions. Host behavior and microhabitat preference might be crucial impediments to symbiont interchanges in cave waters inhabited by more than one *Niphargus* species (e.g., Pozzo dei Cristalli). The installation of two aquaria filled with cave sediment and synthetic cave water has enabled us to maintain distinct *Niphargus* populations ("swimmers" and "crawlers") in captivity for the purpose of future long-term studies on the amphipod – symbiont ecology under controlled conditions.

Notes

Mollusks of the Frasassi karstic complex and adjacent sulfidic spring

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Following the discovery of hydrobiid shells in the remains of subfossil eels found in 2005 at Lago delle Anguille, in the inner part of the Grotta del Fiume at Frasassi (Mariani et al., 2007), we have undertaken a systematic search for this small gastropod in other parts of the cave complex and in the sulfidic spring on the right bank of the Sentino River, which is located in the immediate vicinity of the cave entrance. Eight of the 11 mollusks species identified from empty shells in the sulfidic spring are not typical of sulfidic or creno-stigobiontic environments but they belong to the local riverine malacofauna probably transported over to the sulfidic spring site along with alluvial sediment by the flow of the Sentino River. On the other hand, live specimens of *Potamopyrgus antipodarum* (Gray, 1843), a small, highly-adaptable hydrobiid gastropod with an elongated conical shell some 3.4-6.6 mm high, often with a characteristic keel, were found in the sulfidic spring, and also at Lago Verde, the sulfidic phreatic pool closest to the entrance in the Grotta del Fiume. *P. antipodarum* is an exotic yet invasive species introduced in Italy from New Zealand in 1961. In a few decades, it has colonized springs, streams and rivers throughout the whole length of the Italian peninsula (Favilli et al., 1998; Cianfanelli et al., 2007). In some cases, and especially in the first decades after its appearance in Italy, thanks to its adaptability to different environmental conditions and its partenogenic capability, *P. antipodarum* has formed very dense populations counting thousands of individuals per square meter, thus entering into competition with numerous other fresh water mollusks. This species has not invaded the innermost phreatic lakes of the Frasassi cave complex yet, but its potential in colonizing also peculiar or extremophile environments, such as thermal or subterranean waters, could put the existence of delicate and sensitive endemic hydrobiids at serious risk.

Haitia acuta (Draparnaud, 1805), another alien species introduced in Italy at the end of the 1800's, which is easily recognized from its ovate sinistral shell, is almost consistently present in the sediment of the sulfidic spring. One empty shell was found in a sediment sample from Lago Verde. Thus there is no evidence that this species has colonized the hypogean environment of Frasassi, and the finding of one empty shell at Lago Verde may simply be the result of contamination from the exterior.

Of great interest is the presence, in the phreatic environment of Frasassi, of the small and peculiar hydrobiid belonging to the genus *Islamia*. This genus is diffused throughout southern Europe and in the Mediterraean area, and is represented by numerous species, At least nine *Islamia* species are present in Italy, which can be recognized, with some confidence, only by anatomical analysis of the genital system. Some of them, such as *Islamia* sp.2, have not been officially defined yet although they were reported and described by Giusti et al. (1981) and Bodon & Cianfanelli (2002) in a few localities in the central Apennines (Bodon et al., 2005). Sarbu et al. (2000) report the same species from the Grotta Solfurea (a small cave of the Frasassi complex) but erroneously identified it as *Islamia pusilla*. The shell of *Islamia* sp.2, with a maximum diameter varying between 1.01 to 2.16 mm, and a height between 1.03 and 2.67 mm, is conical-valvatiform, rather robust, waxy and translucent when fresh. The elevated spire is made of 3-3 ¾ rapidly growing convex, dextral whorls, deeply sutured, and with the terminal portion barely or not all descendent near the aperture. The aperture is circular, slightly pyriform and prosocline. The peristome, continuous, scarcely thickened, and barely reflected on the columellar rim, is in contact with the last whorl, or slightly separated from it. The umbilicus is rather narrow, about 1/7-1/2 of the maximum diameter of the shell. The surface of the protoconch exhibits a strong and dense malleated sculpture, characterized by pits with numerous pores and irregular small holes. The surface of the teleconch is smooth, with barely visible growing striae. The operculum is corneous, with a light yellowish color, paucispiral, slightly thicker in the center, without any structure arising from the center of its inner face.

The body of epigean specimens may exhibit a blackish pigmentation more pronounced in the head, along the rim of the foot, and in the mantle, or it may be depigmented with just traces of pigmentation only in the visceral sac. Complete depigmentation is rare. Live specimens from the Frasassi cave exhibit, in the inner part of the otherwise transparent body, an intense pink pigmentation, which is not preserved in samples fixed in ETOH or glutaraldehyde. The base of the tentacles normally contains small ocular spots, which sometimes are absent.

The male genital apparatus, as in other species of the genus *Islamia*, is characterized by an elongated penis, sometimes faintly pigmented, with a pointy apex lacking a filament. A large round lobe, with an internal refracting aspect, juts out on the left side of the penis, in a subapical position or just slightly recessing in respect to the penial apex. Sometimes a weak muscular fold (plica) is visible on the lower surface of the penis, which is rectilinear and not jutting on the left side. The penial duct, within the right side of penis, is sinuous.

The female genital apparatus, as in other species of this genus, is characterized by a well-developed, oval or sacciform proximal receptacle (RS2) with a long canal situated at the end of the "loop", and by a shorter, distal receptacle (RS1) lacking an evident canal.

The stomach lacks a posterior caecum. The intestine has two loops: the first one, with a S shape, runs in contact with the style sac; the second one, which is barely developed, has a U shape, and it extends on the palleal wall. The straight rectal trait terminates with the anal aperture near the palleal border. The osphradium has an oval or reniform shape. The ctenidium is made up of 10-18 well-developed branchial lamellae.

The tenioglossa-type radula is made up of numerous rows of seven teeth, each one with formula: C= 3-4 + 1 + 3-4 / 2 + 2; L= 5 + 1 + 5; M1= 24-25 ca.; M2= 15 ca. The teeth are conform to other species of this genus, but the species *Islamia* sp.2 is characterized by a central tooth with two robust basal cusps on either side.

In summary, our recent studies in the sulfidic lakes of the Frasassi cave complex, and in some hydrochemically similar springs in the Marche, Umbria and Latium regions of central Italy, led to the discovery of a new, small mollusk belonging to the hydrobiid genus *Islamia*, which is temporarily named *Islamia* sp. 2. This gastropod represents an important element in the trophic chain of these ecosystems and is part of the highly specialized biodiversity, which renders Frasassi a unique case of sulfidic karst, both for its geological and biological attributes. However, *Islamia* sp. 2 was present in a sample collected at the sulfidic spring of Frasassi in 1983, whereas it was not found in more recent sampling campaigns in exactly the same site. The recent settlement of another hydrobiid, the exotic and strongly invasive species *Potamopyrgus antipodarum*, may have been the cause of the disappearance of *Islamia* sp. 2. from the Frasassi spring. Moreover, the finding of live specimens of *P. antipodarum* at Lago Verde, within the Grotta del Fiume, causes some preoccupation about the integrity of the endemic hypogean populations as well.

REFERENCES

Bodon M. & Cianfanelli S., 2002 – Idrobiidi freatobi del bacino del fiume Magra (Gastropoda: Prosobranchia: Hydrobiidae). Boll. Malacologico, 38 (1-4): 1-30.

Bodon M., Cianfanelli S., Manganelli G., Pezzoli E. & Giusti F., 2005 – Mollusca Gastropoda Prosobranchia ed Heterobranchia Heterostropha. In: Checklist e distribuzione della fauna italiana. 10.000 specie terrestri e delle acque interne. Mem. Mus. Civ. St. Nat. Verona, 2° Serie: 79-81, 1 CD.

Favilli L., Manganelli G. & Bodon M., 1998 – La distribuzione di *Potamopyrgus antipodarum* (Gray, 1843) in Italia e in Corsica (Prosobranchia: Hydrobiidae). Atti Soc. it. Sci. Nat. Museo civ. Stor. Nat. Milano, 139: 23-55.

Cianfanelli S., Lori E., Bodon M., 2007 – Non-indigenous freshwater molluscs and their distribution in Italy. In Gherardi F.: Biological invader in inland waters: profiles, distribution, and threats. Chapter five: 103-121. Springer, Dordrecht.

Giusti F., Pezzoli E. & Bodon M., 1981 – Notulae malacologicae, XXVIII. Primo contributo alla revisione del genere *Islamia* (Radoman, 1973) in Italia. Lav. Soc. Malac. It., Atti V Conv. SMI: 49-71.

Mariani S., Mainiero M., Barchi, M., van der Borg K., Vonhof H., & Montanari A., 2007. Use of speleologic data to evaluate Holocene uplifting and tilting: an example from the Frasassi anticline (northeastern Apennines, Italy): Earth and Planetary Science Letters, v. 257, p. 313-318.

Sarbu S.M., Galdenzi S., Menichetti M. & Gentile G., 2000 – Geology and biology of the Frasassi caves in Central Italy: an ecological multidisciplinary study of a hypogenic underground karst system. In: Wilkens D., Culver D. & Hamphreys W.F. (Eds.), Ecosystems of the World, Elsevier, New York, pp. 359–378.

Notes

Observations on Niphargus in the Frasassi Cave System

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Gammaridean amphipods of the genus *Niphargus* are widely distributed throughout Europe, with different endemic species resident in almost every freshwater hypogean and interstitial environment (Fišer, *et als*.). They are the dominant aquatic macrofauna in the Frasassi cave system. This presentation compares what we have learned about Frasassi *Niphargus* over the past three years with *Niphargus* species from other areas, focusing on characteristics which allow them to exist under the specialized environmental constraints of the Frasassi cave system.

So far observations suggest that the life history of Frasassi amphipods is similar to most *Niphargus* species. There are more females than males. Males seem to be smaller, and their second gnathopods about the same size as the females'. In contrast, the second gnathopods of most epigean gammarid males are larger than the females (Borowsky 1984, for example). While precopulatory mate-guarding is almost universal in epigean species (Conlan, 1991), it has not been observed in Frasassi or in other *Niphargus* species (Ginet, 1960, for example). This may explain the lack of sexual dimorphism in the second gnathopods, as these are typically used by males to defend females during mate-guarding (Borowsky, 1984) In addition, there seems to be a reproductive rest period between broods in Frasassi and other *Niphargus* species (personal observation and Ginet, 1960). The broods of females in one cave have fewer and larger eggs than a comparable epigean species, but similar to other *Niphargus* species (Borowsky, 1986 and Ginet, 1960, respectively).

Limited observations on reproductive activity show that there are brooding females and juveniles in some Frasassi cave pools, but not others. This may be due to sampling error (collections are limited), to differences in water chemistry requirements for breeding vs. non-breeding females; but more interesting, other investigators have concluded that there may be as many as three different species in the caves (G. Karaman and C. Fišer, personal communication), so it is possible that different pools have different resident species. This remains to be investigated further.

Most importantly for the Frasassi cave ecosystem, however is that endemic *Niphargus* may be employing the caves' chemoautotrophic bacteria as their principal food source (Sarbu *et als*, 2000 and Borowsky, personal observation). Different gammarid species have different food preferences, but, in practice, their complex mouthparts permit them to ingest most organic material (Mayer, 2009). In contrast, behavioral observations of animals from Frasassi's Lago Verde showed that these animals consumed nothing but bacteria-rich fine sediment from the pool.

One incidental but interesting finding is that Frasassi amphipods respond to light even though they lack eyes. Frasassi amphipods do not exhibit a dorsal light reflex. However, when exposed to a broad-spectrum of wavelengths, the level of activity increases significantly. Kureck (1964) found that another Niphargus species (*N. aquilex)* responds to light as well.

In a follow up study, we amplified a portion (303 base pairs) of a long wavelength rhodopsin from Lago Verde *Niphargus*. Further study will use probes made from this sequence to determine the patterns of expression within the animals.

REFERENCES

Borowsky, B. 1983. Behaviors associated with tube-sharing in *Microdeutopus gryllotalpa* (Costa) (Crustacea: Amphipoda). *Journal of Experimental Marine Biology and Ecology* 68: 39-51.

Borowsky, B., l984. The use of the males' gnathopods during precopulation in some gammaridean amphipods. *Crustaceana* 47(3): 245-250.

Borowsky, B., 1986. Laboratory observations of the pattern of reproduction of *Elasmopus laevis*. *Marine Behavior and Physiology.* 12: 246-256.

Conlan, K., 1991. Precopulatory mating behavior and sexual dimorphism in the amphipod Crustacea. *Hydrobiologica* 223(1):255-282.

Fišer, C., P.Trontelj**,** and B. Sket, 2009. http://niphargus.info/introduction/

Ginet, R. 1960. Ecologie, ethologie et biologie de *Niphargus* (Amphpodes Gammarides hypoges). *Annales de Speleologie* 15(2): 241-376.

Kureck, A., 1964. Light Sensitivity in the Amphipod *Niphargus aquilex Schellenbergi* Karaman. *Cellular and Molecular Life Sciences* 20(9) 523-524.

Mayer, G., G. Maier, A. Maas, and D. Waloszek, 2009. Mouthpart morphology of *Gammarus roeselii* compared to a successful invader, *Dikerogammarus villosus* (Amphipoda). *Journal of Crustacean Biology,* 29(2): 161-174.

Sarbu, S.M., S. Galdenzi, M. Menichetti, and G. Gentile, 2000. Geology and biology of the Frasassi Caves in central Italy: an ecological multi-disciplinary study of a hypogenic underground karst system. Chapter 19, in: Ecosystems of the World. H. Wilkens, D. C. Culver, and W. F. Humphries, Eds. Elsevier, New York, pp 356-378.

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Cave Biology and the Replicated Evolutionary Experiment

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Evolutionary biology is largely an observational science. As such, it usually lacks the power of the replicated laboratory experiment. Many evolutionary developments of great importance in the history of life were unique events (e.g., the amniote egg, feathered flight, vertebrate tetrapody, etc.) and hypotheses about the driving mechanisms cannot be tested by replication. It is in this regard that the study of the evolution of cave animals is particularly valuable. The cave environment presents similar challenges to most of the species that evolve cave adaptations. The most important environmental variables are lack of light and energy restrictions. Under these conditions, cave animals typically converge on a suite of phenotypes that include loss of eyes and melanin pigmentation, enhancement of other senses, and metabolic changes. Thus, each cave species is the result of an evolutionary replicate experiment that asks what happens when the lights go out and food is limited. This aspect of replication allows the testing of hypotheses obtained from studying one population by examining other populations and species. This approach is illustrated by genetic studies on eye loss and pigmentation regression in the blind Mexican tetra, *Astyanax mexicanus.*

Quantitative Trait Locus (QTL) analysis of the Pachon cave population revealed 12 different gene loci that were responsible for eye regression and 13 QTL that were involved in melanophore pigment cell loss. While similar numbers of genes affected the two systems, the genetic effects of the cave allele were consistent for eye loss (i.e., cave allele = smaller eye) but variable for pigment cell loss (i.e., cave allele = fewer or greater numbers of pigment cells). This difference in pattern led to the hypothesis that eyes decreased in cave fish because of direct selection against them while pigment cell number decreased because of mutation pressure and genetic drift. This hypothesis makes two predictions that can be tested by looking at replicate populations of cave fishes. The first prediction is that QTL analysis of other populations of *A. mexicanus* will reveal the same differences in polarity between eye and pigmentation QTL. The predicted pattern was observed in replicate QTL experiment on two different cave populations. The second prediction is that eyes should be lost faster than pigmentation in cave fishes because selection can change gene frequencies more quickly than genetic drift. This prediction was tested and supported by comparative analysis of eight species of SE Asian Balitorids, five of which are cave adapted. Thus, the power of replication permitted the testing of the original hypothesis and the results provided support for it. This approach may be applicable to studies of the multiple species of crustaceans, including amphipods, copepods, and ostracods that inhabit the giant Frasassi cave system and other karstic hypogean environments throughout Europe.

Notes

The protozoan ciliates of the Frasassi caves: a potential source of bioactive molecules for biotechnological applications

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The sulfide-rich Frasassi cave complex (Genga, AN, Italy) hosts a still largely uncharacterised microbiota whom study might offers an intriguing view on the solutions adopted by the different species to survive and interact with each others in a such harsh environment. In the frame of a project of Marche Region, we started a preliminary investigation on the species diversity and community structure of protozoan ciliates presents into two main lakes of the Frasassi caves: "Lago Verde" and "Lago Claudia". To date three ciliate species have been collected and identified by means of both morphological and molecular analyses, in the aforementioned lakes: *Coleps hirtus*, *Euplotes aediculatus* and *Urocentrum sp.* A fourth species, *Climacostomum virens* was collected from the sulfidic spring "Sorgente sulfurea libera" of the Sentino River which runs through the Frasassi Gorge. *C. hirtus*, *C. virens* and *E. aediculatus* were gradually adapted to growth under the laboratory conditions, however, we were unable to culture *Urocentrum sp.* that seem to be strictly adapted to the peculiar aquatic habitat of the caves characterized by stable temperature, absence of light and sulfidic (H_2S-rich) water. Moreover, since that ciliates have been shown to be able to synthesize a large variety of bioactive molecules such as, water-soluble cell signaling proteins (i.e. pheromones) and/or various toxins (secondary metabolites) which are mainly used for chemical defence against predators, we assayed the capability of the identified protozoan species to produce such molecules. To this respect, *E. aediculatus* was found to be able to synthesize and constitutively secrete mating-type specific proteins (pheromones) into its extracellular medium. *C. hirtus* was observed to be able to ward off potential predators by discharging ethanol-soluble substance/s that was toxic for a panel of various organisms. With regards to *C. virens,* it is already known that this species is able to discharge a well-characterized molecule called climacostol which posses interesting bioactive properties. Indeed climacostol belongs to the group of natural compounds denoted as resorcinolic lipids (or alkylresorcinols), that have attracted the attention of some researchers for their antimicrobial, antiparasitic, genotoxic, and antitumor activities. Recent studies on antitumor proprieties of climacostol have demonstrated that this molecule effectively and specifically inhibited the growth of different lines of human cancer cells by inducing programmed cell death, while non-tumor cells were not affected by the molecule (Buonanno et al., 2008, Chem.-Biol. Interact. 176: 151-164). We are currently characterising these novel molecules and in a next future we foresee to evaluate their biotechnological potentialities especially for biomedical applications.

Notes

The *Niphargus* symbiosis from the Frasassi Cave ecosystem: a case of multiple partners?

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Sulfide-rich limestone caves such as Frasassi (Italy) and Movile (Romania) contain ecosystems supported entirely by chemoautotrophy. Initial descriptions of these terrestrial chemoautotrophic ecosystems described them as analogs of marine vents and seeps, with an important exception: the lack of symbiotic organisms. Invertebrates with chemoautotrophic symbionts are ubiquitous at marine sulfur-rich environments. In this study, we report that *Niphargus* amphipods, the numerically dominant macro-invertebrates of Frasassi caves, are in fact symbiotic. Using a combination of 16*S* rRNA gene sequencing, fluorescence in situ hybridization (FISH), ^{13}C labeling, and secondary ion mass spectrometry (SIMS), we found that amphipods throughout the cave system were colonized by filamentous bacteria belonging to a single phylotype of the sulfur-cycling clade *Thiothrix*. Moreover, 16*S* rRNA analysis of *Niphargus* gut contents revealed a possible second symbiont: a Mollicute that is closely related to a gut biont of *Rimicaris exoculata*, a symbiotic shrimp found at mid Atlantic hydrothermal vents. Conditions supporting chemoautotrophy likely commenced in the Frasassi cave complex between 350,000 and 1 million years ago. Therefore, the *Niphargus* symbiosis is substantially younger than the ten- to hundred-million year old marine analogs, and could provide valuable insight into the initiation and early evolution of chemoautotrophic symbioses.

Notes

Biological controls on detailed sulfur cycling and the formation of the Frasassi caves

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The Frasassi caves in central Italy are the product of sulfuric acid speleogenesis, where acid derived from the oxidation of dissolved hydrogen sulfide dissolves calcite minerals, often precipitating gypsum as part of the process. The most likely source of the significant hydrogen sulfide that has caused the formation of the Frasassi system is from the Triassic Burano anyhydrite/dolomite sequences. The intercalated gypsum and organic-rich dolomite layers of the Burano formation provide a rich source of the sulfate and organic matter required for bacterial sulfate reduction (BSR), as pointed out by Galdenzi and Maruoka, 2003 and Macalady et al., 2007. While thermodynamically sulfate and organic material such as kerogens should react to produce hydrogen sulfide, the rate of the reaction is very slow abiotically below temperatures of at least 150ºC (commonly known as thermochemical sufate reduction, TSR), and BSR is limited to temperatures below approximately 120ºC (Goldstein and Aizenshtat, 1994; Goldhaber and Orr, 1995; Druschel et al., 2002). Hydrologic pathways governing recharge and fluid flow through the units in the Frasassi area are likely fracture and karst controlled, and in similar systems for these units in the Apennines (e.g. Capaccioni et al., 2001) there is no evidence for these waters getting to depths and temperature significant enough for TSR to take place. Likely hydrologic pathways and chemical kinetics arguments support H_2S formation via BSR, this dissolved hydrogen sulfide is then transported to the water table where it can interact with atmospheric oxygen.

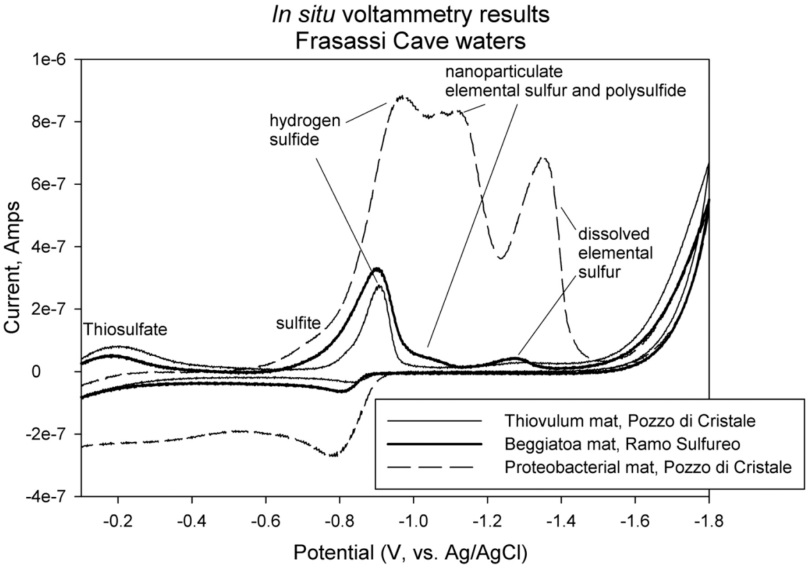

Figure 1 - Example voltammograms from selected areas in the Frasassi cave system illustrating the different forms of intermediate sulfur and hydrogen sulfide present.

Hydrogen sulfide oxidation is accelerated by a variety of microbial species, who utilize hydrogen sulfide for their metabolism. However, sulfide is not completely oxidized to sulfate by all microbial or abiotic processes likely active in the system and a number of dissolved and precipitated sulfur intermediates are present. We have utilized *in situ* voltammetric analyses (Figure 1) and microscopy to characterize the presence of thiosulfate, sulfite, polysulfide, and elemental sulfur associated with waters where active oxidation of H_2S by microbial communities is taking place.

Thus there is a complex set of biotic and abiotic reactions controlling sulfur cycling in this system; variations in any reaction may affect the sulfur isotopic dynamics of the system, and also may have an effect on the microbial ecology and associated rates of sulfide oxidation and speleogenesis. We will review the sulfur cycle from dissolution and BSR formation of sulfide to the oxidation reactions pertinent to sulfide oxidation and cave formation.

REFERENCES

Capaccioni, B., Didero, M., Paletta, C., and Salvadori, P., 2001: Hydrogeochemistry of groundwaters from carbonate formations with basal gypsiferous layers: An example from the Mt. Catria-Mt. Nerone ridge (Northern Appennines, Italy). Journal of Hydrology, Vol. 253, p. 14-26.

Druschel, G.K., Labrenz, M., Thomsen-Ebert, T., Fowle, D.A. and Banfield, J.F., 2002: Biogenic Precipitation of Monomineralic Nanocrystalline Sulfides: Implications of Observed and Modeled Processes to Ore Deposition. Economic Geology Vol. 97, No. 6, p.1319-1329.

Galdenzi, S., and Maruoka, T., 2003: Gypsum deposits in the Frasassi caves, central Italy. Journal of Cave and Karst Studies, Vol. 65(2), p. 111-125.

Goldhaber, M.B. and W.L. Orr, 1995, Kinetic controls on thermochemical sulfate reduction as a source of sedimentary H_2S. In: Vairavamurthy, M.A. and M.A.A. Schoonen (eds): Geochemical transformations of sedimentary sulfur. ACS symposium Series 612, p. 412-425.

Goldstein, T.P., and Z. Aizenshtat, 1994: Thermochemical sulfate reduction. Journal of Thermal Analysis, v. 42, p. 241-290.

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A possibly opportunistic clitellate annelid belonging to the genus *Potamothrix* (family Naididae) in the microbial ecosystems of the Frasassi caves (Italy)

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Mass occurrences of small oligochaetous clitellates in bacterial mats of the Frasassi caves (Regione Marche, Italy) prompted an investigation of the taxonomical identity of these worms. The wet films of sulfur bacteria on the walls and bottoms of parts of the cave system were at some occasions found to contain enormous numbers of a small species of Naididae. However, at other times of exploration, the worm population appeared to have disappeared from these sites in the cave. Naidid oligochaete species are normally identified by the appearance of their internal sexual organs, but in this case none of the worms sampled and examined showed any sign of development of genital structures. Instead some worms appeared to be fragmented individuals with newly healed ends. This suggests that the Frasassi population is not amphimictic (sexually reproducing) but an architomic asexual form.

The Frasassi worms have a chaetal arrangement shared with several common and widely distributed taxa of naidids belonging to the subfamilies Rhyacodrilinae and Tubificinae; their dorsal bundles contain both hair and pectinate chaetae, while their ventral bundles comprise bifid chaetae only (i.e., chaetae with double-pronged tips). However, the specimens do not contain the numerous cellular bodies (coelomocytes) commonly occurring in the coelom of the members of Rhyacodrilinae, which indicates that they are tubificines of some sort. Thus a DNA assessment of the Frasassi population, analyzing both mitochondrial (16S, COI) and nuclear genes (the ITS region), was undertaken, and the sequences were compared with the corresponding data of a number of other tubificines.

16S rDNA sequences showed that the Frasassi worms with high probability is a member, or at least a close relative, of the genus *Potamothrix*. Further, their COI mtDNA (which showed 0% variation among seven sequenced worms) was compared with the corresponding sequences of individuals of other identified or unidentified taxa within *Potamothrix*, collected from other parts of Europe. This comparison demonstrated 14-19% distances to the five nominal species, *P. bavaricus, P. bedoti, P. hammoniensis, P. moldaviensis* and *P. vejdovskyi*, but only 5-6% distances to worms sampled from two different populations in Denmark (River Moelleaa) and Sweden (Lake Lången, Vårgårda). These latter populations also differ by 5% from each other, and so far no sexually mature specimen has been sampled from them, but they both have hairs and pectinate chaetae in their dorsal bundles.

A sixth nominal species, *P. heuscheri*, has previously been recorded from Sweden as well as many other countries, including Italy. It has the chaetal characters of the Frasassi, Moelleaa and Lången populations, but is, on the other hand, regarded as a sexually reproducing taxon. It is one of the most opportunistic freshwater naidids known in the world, e.g., being tolerant to both anoxia and salt water.

It is likely that the Frasassi worm as well as the Scandinavian forms mentioned are, if not identical to, at least closely related to *P. heuscheri*. However, considering the lack of observations of sexually mature individuals, it is possible that all these forms represent a new, or several new, "species" within *Potamothrix*. Species delimitation may here be a matter of opinion, as all species concepts – except morphology-based one – have problems with asexual (clonal) lineages. Sexual species, which, e.g., can be defined as a "separately evolving metapopulations" (De Querioz, 2007) are easier to deal with, and they can be delimited by applying a combinations of criteria (genetic and morphological separation, evidence of ceased gene flow, reproductive isolation, etc.). With few exceptions, mitochondrial DNA (such as COI sequences) is maternally inherited only. Therefore, great COI differences may still occur within an amphimictic population. Nuclear genes, on the other hand, evolve as the result of continuous recombinations of male and female DNA, and ribosomal genes (such as ITS) tend to get homogenized over time (by concerted evolution). Thus a ITS gene tree can often be used as a good indicator of the species tree. In clonal animals, were there no longer is any gene flow between lineages, mitochondrial as well as nuclear gene trees can be used to infer genetic differentiation, but the number of lineages that fulfill the requirement of separate evolution (i.e., number of species) is virtually indefinite.

The ITS sequences of three Frasassi and two Lake Lången specimens were compared with each other, and with those of *P. bavaricus* and *P. hammoniensis*. The first five specimens mentioned were similar, with only a few base substitutions in the individual worms, but the Lången worms have shorter ITS than those from Frasassi. This is due to two consistent gaps, one located in ITS 1 and 63 base pairs long, the second in ITS 2 and 2 base pairs long, but it is possible that the number of events (deletions or insertions) responsible for these gaps is very low. Nevertheless, this means that there is mitochondrial as well as (limited?) nuclear support for these two populations being separately evolving.

In conclusion, the Frasassi *Potamothrix* worms are likely to be members of a whole complex of clonal lineages, with some probability closely related to the widely distributed and highly opportunistic, but sexually reproducing, *P. heuscheri*. If this complex proves to be obligatorily asexual, it may deserve the status of a new nominal species, separated from the latter. Should there be gene flow between some of these lineages, but not with *P. heuscheri* s. str., it may even be possible to recognize several distinct, but separately evolving species within the complex. However, much more information on the various populations of *Potamothrix*, preferably world-wide, is needed, before any such formal taxonomic revision can be undertaken.

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Subterranean assemblages of the genus Niphargus: the role of functional morphology

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The studies of subterranean communities consist of local checklists, definitions of species' niches using physical and chemical parameters and interspecific interactions studies. Apparently the latter importantly influence subterranean communities, i.e. competition and predation have been shown to affect microdistribution of different species within a single locality. Species' specialization might result in niche separation and reduction of negative interspecific interactions to minimum. Niche characterization using physical and chemical determinants of environment might fail in cave animals for several reasons. Alternatively, linking adaptive morphology with habitat use can illuminate the phenomenon of niche partitioning in caves.

In European genus *Niphargus* two or more species may co-exist in one place. Here we revise morphology in six interstitial and seven cave communities comprising minimally three niphargid species. The underlying hypothesis was that similar morphological patterns emerge in different localities because of limited resources in subterranean habitats. We reviewed a set of 13 functional characters. The principal component analysis identified three (stout-slender, short-long legs, small-large) and two principal components (stout-slender, short-long legs) in interstitial and cave communities, respectively. Although we found no distinct morphological groups are individual species from the same localities scattered across the PCA plots. Interstitial communities consist of slender and stout individuals, where surrounding sand grains constrain the variation of appendage lengths. Caves are obviously more heterogeneous. Detailed analysis of two the species-richest cave systems suggest that cracks in the rock invade small stout, small slender and large slender specimens, river flows invade mid to large slender specimens with short appendages while deep waters with still water harbor large stout specimens with long appendages. Morphologically different species co-exist in the same water bodies, while similar species are spatially separated.

Notes

What lies beneath: diversity, ecology and evolution of stygobiotic copepods (Crustacea, Copepoda)

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The subclass Copepoda virtually colonized all kinds of aquatic habitats, from the deep-sea hydrothermal vents, semiterrestrial habitats (e.g., leaf litter in tropical and temperate areas), phytotelmata, and successfully invaded inland waters. They are ubiquitary in fresh water, from plankton to benthos of lenthic (e.g., lake, ponds, pools, limnocrene springs) and lothic environments, where they predominantly live linked to stream and river beds, as epibenthic and, more frequently, among the minute grains of the interstitial environment. With a great success they entered ground water, showing a high diversification in this vast environment. They are common inhabitants of springs, which represent "open windows" to the *underground realm*, and are frequently found in different cave habitats, in the epikarst, vadose and phreatic zone of karstic aquifers, where they live as free-swimmers in the planktonic environment, or alternatively in the hyperbenthic habitats of small concretional pools or deep phreatic lakes. Another habitat widely colonised by groundwater copepods is represented by the minute voids among sand and gravel grains of unconsolidated aquifers, in both hyporheic and deep alluvial groundwater. Copepods entered groundwater in different times and via different evolutionary pathways, and this condition is reflected in the wide diversification observable among stygobiotic copepods. Four free-living copepod orders have successfully invaded fresh ground water; namely: Calanoida, Cyclopida, Harpacticoida and Gelyelloida and members of each order show diverse habitat and microhabitat preferences, as reflection of different preadaptive features, already present in their respective epigean ancestors. Is there a groundwater habitat that copepods were unable to colonise? A challenge in a challenging environment: the discovery of stygobiotic copepods in the sulfidic lakes of the Frasassi cave system. Preliminary results are presented on the taxonomic diversification of planktonic copepods in this extremophile chemoautotrophic environment.

REFERENCES

Brancelj A. & Dumont H.J. (2007) A review of the diversity, adaptations and groundwater colonization pathways in Cladocera and Calanoida (Crustacea), two rare and contrasting groups of stygobionts. *Archiv Hydrobiol.*, 168: 3-17.

Deharveng L., Stoch F., Gibert J., Bedos A., Galassi D.M.P., Zagmajster M., Brancelj A., Camacho A., Fiers F., Martin P., Giani N., Magniez G. & Marmonier P. (2009) Groundwater biodiversity in Europe. *Freshwat. Biol.*, 54: 709-726.

Galassi D.M.P. (2001) Groundwater copepods (Crustacea: Copepoda): diversity patterns over ecological and evolutionary scales. *Hydrobiologia*, 453/454: 227-253.

Galassi D.M.P., Huys R. & Reid J.W. (2009) Diversity, ecology and evolution of groundwater copepods. *Freshwat. Biol.*, 54: 691-708.

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The hypogene caves of the Apennines, with special regard to the Frasassi karst

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The Apennine hypogene caves

Hypogene caves are quite common in the Apennines. Reasearch, however, has not had the same development in the whole area. Some caves are well known, while the characteristics of others are only outlined, and it is probable that in some zones hypogene caves are more diffuse than presently believed. The oxidation of H_2S is the most important chemical process for hypogene speleogenesis in the area, and some caves represent good places for the study of active corrosional processes in many different biological and geological aspects. Most caves, however, are relict, and the past flow of sulfidic water is recorded by typical morphologies and gypsum deposits.

Hypogene caves are mostly located in small limestone outcrops, covered by low-permeable units, where the sulfidic water rising from the depth can reach an oxidizing environment or mix with descending freshwater. Some hypogenic caves, however are known also in open hydrogeologic massifs, where epigenic caves prevail. The wide variety of examples, developed in different geological settings, makes the Apennines a good place to evaluate the influence of geologic and geomorphic factors on the cave enlargement and morphology. There are both phreatic and water table hypogene caves: pure phreatic caves typically develop when karst evolves below the water table in structures almost completely covered by low-permeable units (Acquasanta Terme, Monte Cucco, Serra del Gufo). Water table caves are known as well where a direct recharge of freshwater can reach the sulfidic ground-water from the karst surface (Frasassi, Pozzi della Piana, Sant'Angelo caves).

The hypogenic caves have generally a multistage evolution, related to the progressive deepening of the surface streams and of the related base level. A phreatic cave can evolve to a water table cave because of the erosion of a pre-existing low-permeable cover through time. The widening of the limestone outcrops and the enlargement of fissures in the vadose zone, in fact, increase the direct recharge of freshwater, favoring sulfide oxidation near the water table. Horizontal passages become prevalent over inclined ones, morphologies and deposits related to condensation corrosion (like wall pockets and gypsum deposits), lacking in the wholly phreatic caves, can develop in partly flooded rooms and passages.

Active speleogenetic processes due to H_2S oxidation can be directly observed in different hydrogeologic settings: in highly permeable aquifers with easy recharge of freshwater (Frasassi caves), in thermal caves, below low-permeable cover (Acquasanta Terme), or in marine thermal caves, with salt water intrusion (Capo Palinuro).

Frasassi caves

The Frasassi caves are the most studied hypogene caves in Italy; sulfuric acid speleogenesis is active in the lowest level, while relict features and deposits due to the past flow of sulfidic water characterize the upper, dry levels. The caves reach more than 25 km in total length and open along the steep walls of the Frasassi Gorge, cut in Lower Jurassic limestone. The caves consist of a network of ramifying, mainly sub-horizontal passages in which wide rooms (up to ~106 m^3) alternate with smaller tubes (Figure 3); they are organized in some superimposed and interconnected levels, the genesis of which is related to the different steps in the lowering of the base level.

The sulfidic groundwater is fed by a combination of rising sulfidic water and meteoric seepage water. The sulfidic waters are cold and rich in Ca^{2+}, Na^+, and HCO_3^-, Cl^-, $SO_4^=$ and have high H_2S content (up to 18 mg/L). Significant differences exist in the chemistry of phreatic water inside the cave. Low water flow causes seepage water to remain on the surface due to its lower density across a wide zone of the cave. On the contrary, water rising from a deep zone of the aquifer directly feeds some pools of the cave. The groundwater chemistry varies throughout the year, as it is controlled by the mixing ratio between meteoric and sulfidic waters, with a percentage of seepage water that at the springs varies from 30 to 60% in the different seasons. The highest concentrations (about 2 g/L) occur at the end of summer, that represents the dry season. H_2S and CO_2 are released into the cave atmosphere from the water and can diffuse into nearby rooms; their concentration in the air undergoes to seasonal changes, with opposite trends. H_2S concentration depends directly on water dilution, while CO_2 in the air increases during the same period.

The flowing water is aggressive as regard to limestone, and the release of H_2S and its oxidation in the air causes active formation of white, micro crystalline, slushy gypsum on the cave walls, associated with small amounts of elemental sulfur in places. Some large crystals grow on the gypsum replacement crusts or directly on the limestone. The limestone surface under the gypsum crust is severely corroded, with hemispheric corrosion pockets a few cm deep. The intensity of these reactions has been measured using limestone tablets with known surface area and mass. The average weight loss was ~15 $mg/cm^2/yr$ in both the groundwater and the atmosphere. This corresponds to a dissolution rate of ~0.05 mm/yr on the limestone surface.

The cave development occurred mainly in the shallow phreatic zone, where rising H_2S-rich ground-water mixed with oxygenated seepage water. In large zones wide water-air interfaces maintained for long periods, and condensation corrosion due to H_2S and CO_2 could play an important role in the cave growth. The non-uniform recharge of meteoric water influenced the speleogenesis and cave features. The main rooms in the Grotta del Vento, in fact, are located where a thin marl cover increases the seepage recharge to the sulfidic aquifer (Figure 9). In the highest cave levels, over 250 m above the present water table, gypsum deposition is not known. The general cave setting and the corrosional ceiling channels suggest that cave evolution occurred in prevailing phreatic conditions.

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Metabolic activity of a cyanobacterial mat in a sulfidic spring emerging from the Frasassi cave system, Italy

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Cold sulfidic springs emerge from the Frasassi cave system, Italy, and provide an environment that harbors sulfide oxidizing mats with unusually high biomass of colorless sulfide oxidizing bacteria, anoxygenic phototrophic bacteria and cyanobacteria. We investigated a mat growing in a sulfidic spring with ambient total sulfide and oxygen concentrations of 625 µmol L-1 and 10-15 µmol L-1, respectively. Microscopic observations and hyperspectral imaging showed that cyanobacteria dominated the mat and were closely associated with colorless sulfide oxidizing bacteria. Microsensor measurements showed that, in the light, the cyanobacteria were able to simultaneously perform oxygenic and anoxygenic photosynthesis, which resulted in highly elevated oxygen (~250 µmol L-1) and close to zero sulfide concentrations in the top 1 mm of the mat.

Notes

Metagenomic, phylogenetic, and culture-based analysis of extremely acidic sulfidic cave snottites

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Microbial biofilms called snottites create the most acidic (pH 0-1) environments in the Frasassi cave system. These biofilms are formed by sulfur oxidizing acidophiles that live on $H_2S(g)$ in the cave atmosphere. We used a combination of metagenomic, phylogenetic, and culture-based analyses to investigate the taxonomic composition, ecology and biogeochemistry of snottite communities from throughout the Frasassi cave system. Frasassi snottites are populated by only a few acidophilic bacteria and archaea, and are among the least diverse biological communities known. All snottites sampled to date are dominated by *Acidithiobacillus spp.*, which is a keystone member of the snottite community and is always more than 50% of the total cells. *Acidithiobacillus* strains cultured from snottites are autotrophic sulfur oxidizers, and excrete abundant extracellular polymers similar to those in the snottite matrix. Other snottite microorganisms include heterotrophic archaea (Thermoplasmales group, 0 to 40% of cells) and smaller populations of *Acidimicrobium* species (less than 15% of cells). Rare snottite microorganisms include bacteria of the *Sulfobacillus* and TM6 lineages. Filamentous fungi and protists are also commonly observed. Metagenomic analyses reveal metabolic capabilities of snottite organisms, including nutrient and energy metabolisms as well as different adaptations for survival at extremely low pH.

Notes

The subterranean genus *Niphargus* Schiodte, 1847 (Amphipoda, fam. Niphargidae), taxonomy, origin, and relationships between Italian and Balkan members of this genus

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The subterranean genus *Niphargus* (Schiodte, 1849) (Amphipoda, fam. Niphargidae) is widely distributed in various types of the subterranean waters over central and southern Europe, Asia Minor and Near East with over 200 taxa belonging to several different groups mentioned by various authors as distinct genera, subgenera or just groups of species [*Stygodites*, *Phaenogammarus*; *Protoniphargopsis*; *Supraniphargus*; *Carpathoniphargus*, *Jovaniphargus*, *Orniphargus*, *Karamaniella*, *Martynovia*, etc.].

Originated from the marine blind ancestors (probably of digging type), this genus settled various types of the subterranean waters [in the caves, springs, phreatic waters, deep lakes, etc.]. The body of various species is very different in addition to the ecological niches they settled in, and the type of food they consume.

The members of this genus are in full evolution, very adaptive, still in the process of splitting into different taxa, and with very variable and numerous taxonomical characters, which make the taxonomy of this genus very difficult and problematic.

The comparison of the fauna of genus *Niphargus* in Italy and that of Balkan Peninsula shows many very interesting correlations and leading to various conclusions. Because of the difficulty in collecting these animals, and because the intensive pollution and consumption of epigean and subterranean waters over entire Europe, the living habitat of the species of *Niphargus* is in a high danger of disappearance.

Notes

Enigmatic archaea from the dark, anoxic terrestrial subsurface

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Abstract from: Macalady et al. 2009. Geochimica et Cosmochimica Acta 73 (13, Supplement 1 June 2009): A809.

Cave divers exploring a remote underwater passage in the sulfidic Frasassi cave system (Italy) discovered unusual, rope-like microbial biofilms in anoxic water. Geochemical data suggest that little redox energy is available for life, consistent with low signal from domain-specific FISH probes. The carbon isotope signatures of the biofilm (-33‰) and DIC (-9‰) indicate *in situ* production by lithoautotrophs using RuBisCO. 16S rDNA libraries constructed from the biofilm are dominated by archaea in the enigmatic Marine Benthic Group D (MBG-D/DHVE-1) along with diverse sulfate reducing bacteria. Most of the remaining clones affiliate with one of 11 major uncultivated or novel prokaryotic lineages. Diverse dsrAB gene sequences were retrieved from the biofilm, consistent with high sulfate concentrations and undetectable or extremely low oxygen, nitrate, and iron concentrations. Methane is detectable in the anoxic water although no 16S rDNA sequences associated with known methanogens or anaerobic methane oxidizers were retrieved. mcrA gene sequences retrieved from the biofilm are not related to cultivated methanogens or to known anaerobic methane oxidizers. Our data suggest that novel archaea and bacteria, including MBG-D archaea, are important in the dark, energy-limited cave biofilm. These microorganisms and their potentially novel metabolic strategies are relevant for understanding biogeochemistry and biosignatures of non-photosynthetic, energy-limited environments on the modern and ancient Earth and elsewhere in the solar system.

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Frasassi's microbial sulfur world: Progress and prospects

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Research from 2003 to the present has revealed the incredible beauty and richness of Frasassi's microbial ecosystem. Stable isotope measurements of organic matter in the sulfidic zones, initiated by Galdenzi & Sarbu (Galdenzi and Sarbu, 2000), have confirmed the idea that microbes are the base of a food web isolated from sunlight and organic matter being produced at the earth's surface. Throughout the caves, organic matter is produced from carbon dioxide by bacteria that derive energy by combining hydrogen sulfide and oxygen. Complicated seasonal and spatial variations in mixing between deep sulfidic water and oxygen-rich percolating water contribute to the high diversity of sulfur oxidizing biofilm types, making Frasassi an attractive model system for studying the ecology, behavior and chemical signatures left by sulfur oxidizing groups also found in modern and ancient sulfidic caves (Hose and Macalady, 2006), springs, lakes, marine sediments, and hydrothermal vents. Study of the neutral pH biofilms below the water table showed that turbulence and sulfide/oxygen ratios largely determine which species are present (Macalady et al., 2008; Macalady et al., 2006).

Cave walls above the water table in the sulfidic zones are also covered by microbial biofilms, including near-neutral (pH 6) biofilms known as biovermiculations (Jones et al., 2008), and pH 0-1 snottites constructed by some of the most acid-tolerant microbes known on Earth. Because the snottite communities have extremely few species (Macalady et al., 2007), we are able to investigate their ecology and evolution using cutting-edge genomic analyses. We expect that these analyses will offer key insights into the metabolism of snottite microorganisms, and reveal something new about how microbes evolve when isolated deep underground. Frasassi also boasts an important microbiology first: the discovery of a chemoautotrophic bacteria-animal symbiosis outside the oceans (Dattagupta et al., 2009). Many questions remain. Ongoing work using microsensors [Druschel, MPI Bremen] will enable us to estimate the contribution of microbial activity to limestone dissolution and cave formation.

A new biofilm type discovered in the dark, energy-poor anoxic layer of a remote Frasassi lake contains many microbial species with no close relatives, and offers the opportunity to investigate metabolic strategies that may have been important on the anoxic and pre-photosynthetic Archean earth. Last but not least, the microbial and biogeochemical groundwork that has been established at Frasassi makes it an ideal system for investigating the fundamental behavior of stable isotopes of sulfur [Zerkle] and calcium [Fantle]. Microbial research at Frasassi has succeeded in part because of close collaborations with speleologists, whose skill and dedication to cave exploration outstrip what is possible for full-time research scientists. We hope that this cooperative example lights the way for further important advances in understanding the Frasassi ecosystem and the microbiology and biogeochemistry of caves all over the world.

REFERENCES

Dattagupta, S., Schaperdoth, I., Montanari, A., Mariani, S., Kita, N., Valley, J.W., and Macalady, J.L., 2009, A Recently Evolved Symbiosis Between Chemoautotrophic Bacteria and a Cave-dwelling Amphipod: ISME J, v. 3, p. 935-943.

Galdenzi, S., and Sarbu, S.M., 2000, Chemiosintesi e speleogenesi in un ecosistema ipogeo: i rami sulfurei delle grotte di frasassi: Le Grotte d'Italia, v. 1, p. 3-18.

Hose, L.D., and Macalady, J., 2006, Observations from active sulfidic karst systems: Is the present the key to understanding Guadalupe Mountain speleogenesis?, 57th Annual Field Conference Guidebook: Socorro, NM, New Mexico Geological Society.

Jones, D.S., Lyon, E.H., and Macalady, J.L., 2008, Geomicrobiology of sulfidic cave biovermiculations: Journal of Cave and Karst Studies, v. 70, p. 78-93.

Macalady, J.L., Dattagupta, S., Schaperdoth, I., Jones, D.S., Druschel, G.K., and Eastman, D., 2008, Niche differentiation among sulfur-oxidizing bacterial populations in cave waters: ISME J, v. 2, p. 509-601.

Macalady, J.L., Jones, D.S., and Lyon, E.H., 2007, Extremely acidic, pendulous microbial biofilms from the Frasassi cave system, Italy: Environmental Microbiology, v. 9, p. 1402-1414.

Macalady, J.L., Lyon, E.H., Koffman, B., Albertson, L.K., Meyer, K., Galdenzi, S., and Mariani, S., 2006, Dominant microbial populations in limestone-corroding stream biofilms, Frasassi cave system, Italy: Applied and Environmental Microbiology, v. 72, p. 5596-5609.

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Sulfur isotope geochemistry relating to the Frasassi cave system

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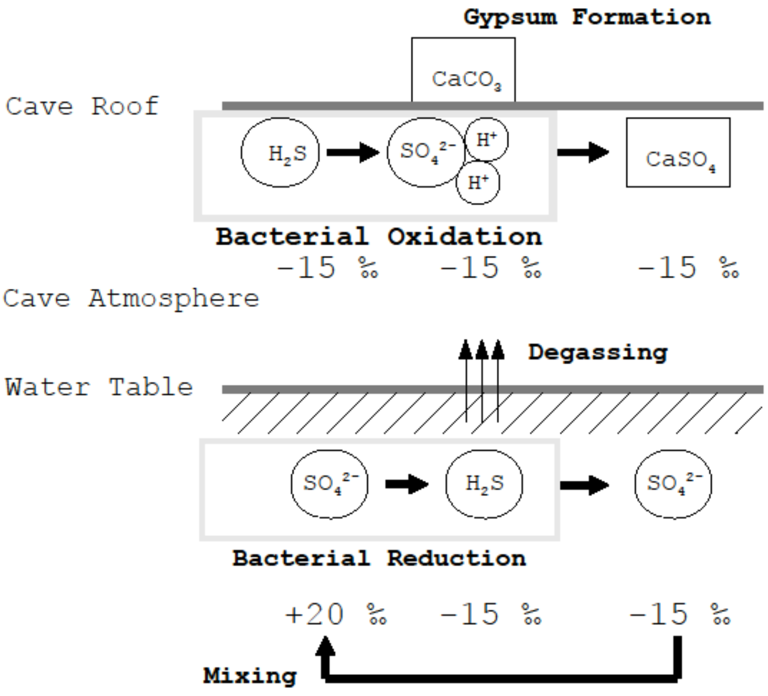
The following sulfur-containing species can be found in the Frasassi cave system; (1) SO_4^{2-} in groundwater, (2) HS^-, S^{2-} in groundwater, (3) H_2S in the cave atmosphere, and (4) gypsum (and a small amount of elemental sulfur) on the cave wall. Using the isotopic composition of sulfur, we can discuss the origin of the sulfur in these species and the processes occurring in the cave system.

(A) Sulfate and sulfide in groundwater

The $\delta^{34}S$ values of sulfate in the Frasassi groundwater generally range between +20 and +22 ‰ (relative to V-CDT). These values are similar to the values found in marine-water sulfate, implying that the sulfate in the groundwater is primarily supplied by dissolution of sulfate minerals in evaporite. In fact, the Triassic evaporitic sequence (Burano Formation), consisting mainly of anhydrite and dolomite, exists under the limestone sequences in which the Frasassi cave is forming.

There is a variation of 2 ‰ for sulfate $\delta^{34}S$ in the groundwater. Two processes are involved in this variation. One process is the bacterial reduction of sulfate. Sulfate-reducing bacteria preferentially use the lighter isotopes (^{32}S over ^{34}S), leaving behind the sulfate that is isotopically enriched in ^{34}S.



The other process is the addition of ^{34}S-depleted sulfate. As mentioned above, the H_2S gases produced by bacterial reduction have a lower δ^{34}S value than that of the original and residual sulfate. The limestone sequences contain sulfide minerals, mainly pyrite, FeS_2, as accessory minerals. These pyrites were produced by the reaction between H_2S and Fe^{2+} on the bottom of the ancient deep sea. The oxidation of these pyrites, possibly by O_2 dissolved in meteoric water, can produce ^{34}S-depleted sulfate. In addition, the dissolution of ^{34}S-depleted gypsum, which we will describe later, can also produce ^{34}S-depleted sulfate and can be induced during migration of the seepage waters.

The δ^{34}S values of sulfide in the Frasassi groundwater range between –14 and –17 ‰. The seasonal variations for each spring are relatively small (within 1 ‰); however, the δ^{34}S values of the spring water are different for each. These differences can be induced by the difference in the isotopic fractionation between sulfate and sulfide during bacterial reduction. This type of isotopic fractionation should be controlled by the environmental factors (especially the temperature of the spring water) where the bacterial reduction occurs.

(B) H_2S in a cave atmosphere and gypsum on the cave wall

Gypsums are observed where the emission of H_2S occurs and often occur as a crust covered on limestone walls above the current water table. These vadose gypsum crusts have □^{34}S values similar to those of H_2S in the spring water, implying the oxidation of H_2S to H_2SO_4 and the reaction between calcite and sulfuric acid occurs above the water table (Fig.1). Gypsums observed where H_2S emission ceased also have δ^{34}S values much lower than sulfate, but slightly higher than the actively forming gypsums. There is a difference of 10 ‰ in the maximum between ancient and present gypsum. A part of this difference is due to the difference in isotopic fractionation during bacterial reduction. The rest of the difference may reflect processes such as (1) the alteration of kinetic isotopic fractionation for H_2S-oxidation, (2) the alteration of sulfate δ^{34}S before the reduction. The δ^{34}S values of sulfate can be altered by the modification of the contributions of seepage waters to the spring water or by the modification of the regions of sulfate sources in the Triassic evaporitic sequence.

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Geologic setting of the Frasassi cave complex: a review by A. Montanari

(from Mariani et al., 2007, Earth and Planetary Science Letters, v. 257, p. 313-318.)

The Frasassi cave complex is located in the core of the Mt. Frasassi-Mt. Valmontagnana anticline, a 300°-120° trending structure of the northeastern Apennines thrust-and-fold belt (Marche Region; Fig.1A-B).

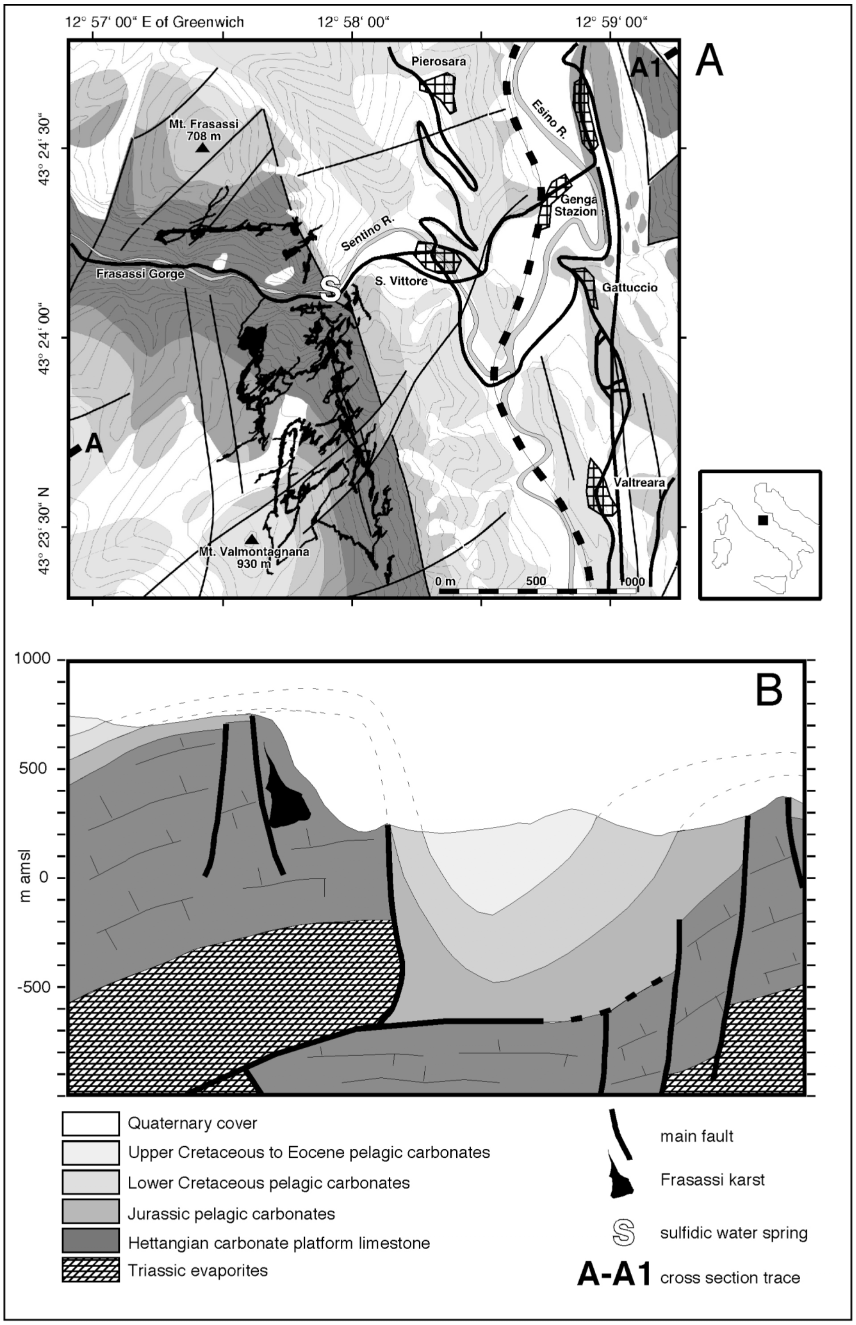

Fig. 1 - A) Simplified geologic map of the Frasassi area, and B) cross section through the Mt. Valmontagnana-Frasassi anticline and the San Vittore syncline (original by the authors)

It consists of a 25-km-long karst maze carved in the Hettangian Calcare Massiccio Formation, a 800-m-thick white, massive, pure carbonate platform limestone. The anticline is cut by the Sentino River, which flows N100°E at the bottom of the 600 m deep Frasassi Gorge, just before merging into the Esino River.

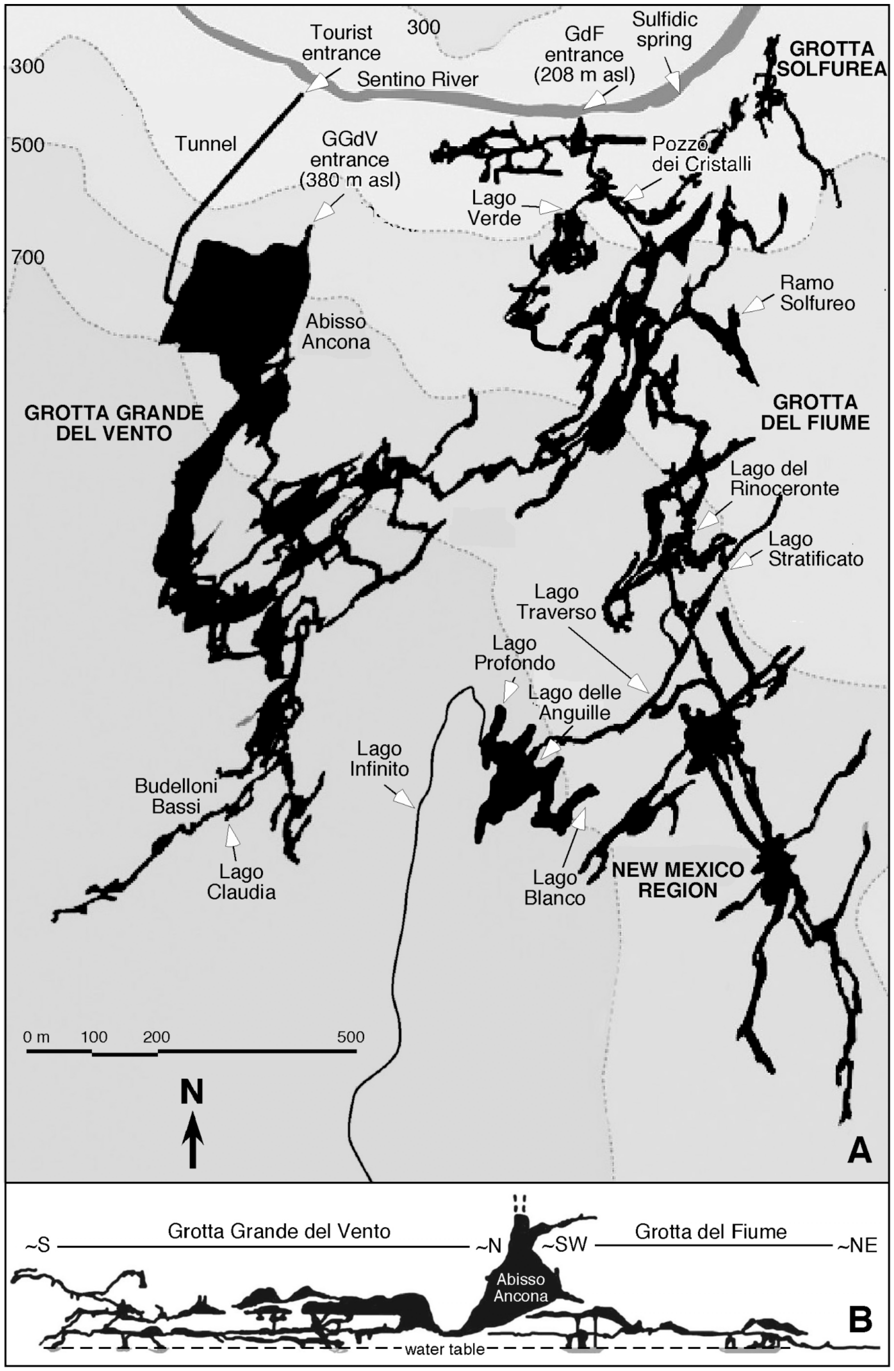

Fig. 2 - A) Map of the Grotta Grande del Vento-Grotta del Fiume cave complex. Note that the long stretch of Lagho Infinito represents a linear transect. The surveying of this region of the cave, which reaches about 1.5 km inside the mountain, is still in progress. B) Idealized cross section of the caves showing the arrangement of horizontal hypogean floors.

The northeastern limb of the anticline is bound by a nearly vertical fault, which represents a paleomargin of a Jurassic-age horst. During the Neogene compressional phase of the Apennine deformation, the horst was folded, uplifted, and displaced to the northeast on a blind thrust [1]. Its eastern steep margin behaved as a bulldozer front, which deformed the adjacent graben multilayer Jurassic carbonate succession, along with the overlying Cretaceous and Tertiary pelagic carbonates to form the tight San Vittore-Pierosara syncline (Fig. 1B). Jurassic to Tertiary thin-bedded limestone formations in stratigraphic contact with the Calcare Massiccio are found at the summits of Mt. Valmontagnana and Mt. Frasassi (Fig. 1A-B).

The Calcare Massiccio rests on top of the Triassic Burano Formation, consisting of up to 2,000-m-thick alternating dolomites, anhydrites, and black shales [2]. This formation is not exposed in the Marche Region, but is drilled in deep boreholes [e.g., 3], and is often manifested by sulfidic (H_2S-rich) water springs. In the Frasassi Gorge these springs are located at the intersection of the riverbed and the bulldozer fault (Fig. 1A), and are found also within the Frasassi caves.

The karst complex at Frasassi is represented by a dozen or so large caves, most of them situated on the southern, Mt. Valmontagnana side of the gorge. Among them, the Grotta Grande del Vento (GGdV)-Grotta del Fiume (GdF) is the largest interconnected system known at present in the northeastern Apennines (Fig. 2A). The second largest cave in the area is the Buco Cattivo, which is almost certainly part of the GGdV-GdF system, although no passage connecting the two systems is known to date.

The karst grid of the Frasassi cave complex developed along a dense network of intersecting fractures and normal faults trending primarily NW-SE and NE-SW, parallel and transverse to the Mt. Frasassi-Mt. Valmontagnana anticline, respectively. A secondary, N-S trending system of probable Jurassic-age fractures and faults is also present in the calcareous anticline core, and it is emphasized by karstic corrosion in the cave development (Fig. 2A).

Seismo-tectonic setting

Since the end of the early Miocene, the tectonic evolution of the Northern Apennines is characterized by regional uplifting, accompanied by contemporaneous eastward migration of coupled compression and extension in the foreland and hinterland, respectively (e.g. [4,5]). The causes of the uplift are a controversial issue. Some authors propose that regional uplift is related in some way to a lithospheric subduction process, which causes bulging of the Apennine lithosphere as an isostatic response to a slab detachment [6,7]. Alternatively, the rise of the Apennines has been explained as a consequence of a long-wavelength mantle uplift [8].

At present, extension is affecting the main Apennines ridge, whilst compression is localized along the Adriatic coast. This pattern is documented by the focal mechanisms of the major earthquakes [9], their hypocenter distribution [10], integrated data on the active stress field (including borehole breakouts, seismicity, and active faults [11]), and by geodetic (GPS) data [12].

This kind of evolution is the base of a regional seismotectonic zoning [13, 14], where the northeastern Apennines are divided into three NW-SE elongated zones (Fig. 3). The inner zone represented by the Apennines Ridge, and the outer Adriatic zone are presently undergoing extension and compression, respectively. These zones are separated by a quasi-aseismic intermediate zone, where at the surface compression is no longer active, while extension has not been activated yet. The major historically and instrumentally documented earthquakes of the region ($5<M<7$) occur in the central part of Apennine Ridge, and their foci are distributed along SW-dipping normal faults (Umbria Fault System, UFS; [15]), antithetic to an east-dipping, low-angle detachment (Altotiberina fault, ATF; [16, 17]). In this dynamic scenario, the Frasassi area is located at the eastern border of this inner extensional zone (Fig. 3A).

In Figure 3B-C we reproduce the pattern of instrumental seismicity, recorded in the last 20 years [10], along a section trending WSW-ENE from Perugia to Ancona, and crossing the Frasassi area.

Two main clusters of hypocenters can be observed below the main ridge of the Apennines: 1) a relatively shallow cluster (depth D = 5-15 km), which is confined above an east-dipping detachment; and 2) a deeper cluster (D = 20-50 km), roughly aligned along a W-dipping trajectory. This pattern is consistent with the proposed seismotectonic zoning in Figure 3A, if we consider the shallow and the deep clusters as expressions of extension and compression, respectively. The Frasassi area is located east of the main shallow cluster of seismicity (corresponding to the UFS), where relatively sparse and mild extensional seismicity occurs at depth D<15 km.

While uplifting rates through the Quaternary can be deduced from the elevation and age of ineterglacial fluvial deposits, tilting, which could be related to the lithospheric bulging and/or (locally) to the rollback of reactivated listric faults, is difficult to estimate from such geomorphologic features. On the other hand, in the Frasassi cave complex we found geomorphic features, such as tilted paleo-horizontal markers and datable material (subfossils and calcite deposits), which indicate the location and age of the water table during the Holocene, and thus allow to estimate, with high precision and accuracy, the recent uplifting rate and tilting of this region.

Speleogenesis and karst evolution

During the Pleistocene, in a combined process of antecedence and superposition [18, 19], the Sentino River carved the Frasassi Gorge through the Calcare Massiccio anticlinal core. Meanwhile, the core of the anticline was karstified with the formation of a large cave complex. The cave architecture is characterized by seven main horizontal "floors", which are connected by narrow, steep or nearly vertical shafts (Fig. 2B). Some large rooms, such as the 250 m high Abisso Ancona (estimated to be about 10^6 m^3 in size), were formed after the collapse of the thin rock diaphragms separating the floors. This floor organization is reminiscent of, and can be correlated with, the fluvial terrace step topography that characterizes the lower Esino valley [20-23]. This is the result of alternating erosion and deposition controlled by successive Quaternary glacial-interglacial phases during a steady, generalized regional tectonic uplift.

The horizontal karst developments probably formed during glacial periods when the gorge was filled with detritus, which, due to the scarce vegetation, moved down from the steep slopes of the gorge clogging the riverbed and raising the water table. Such loose gorge filling would have been removed by fluvial erosion at the beginning of interglacial phases, allowing the Sentino to quickly reach the limestone bedrock and continue its slow process of erosion and deepening of the gorge. The process would restart with the successive glacial period but the water table would not reach the same level as in the previous phase, which, in the mean time, has risen due to the continuous tectonic uplift of the region.

A major role in the Frasassi speleogenesis is played by H_2S-rich water, which wells from the Triassic Burano Formation underlying the Hettangian Calcare Massiccio. The Burano Formation is in fact largely made up of anhydrite ($CaSO_4$), which, when in contact with deep waters, forms gypsum ($CaSO_4 \blacklozenge 2H_2O$). Hydrogen sulfide can be formed in these deep environments when sulfate (sourced from gypsum) is reduced through interaction with organic compounds, a process, which is microbially catalyzed at low temperatures [24-26]. This allows sulfur, in the form hydrogen sulfide (H_2S), to enter into solution with the upwelling groundwater of the aquifer. When the sulfide-enriched groundwater reaches the surface, it comes into contact with oxygen contained in carbonate ground waters, causing the formation of sulfuric acid (H_2SO_4). The sulfuric acid attacks the limestone causing dissolution (corrosion) of calcium carbonate. This chemical reaction can also occur in the cave atmosphere with sulfuric vapors attacking the limestone walls of the cave, causing the formation of microcrystalline gypsum slush masses or, in some cases, spectacular flowery decorations of selenite (i.e., macrocrystalline gypsum) [27]. However, if this reaction takes place under water, the high solubility of gypsum prevents the formation of gypsum deposits, and the reaction results in dissolving the calcite of the limestone bedrock, with the consequent formation of caves [27, 28].

Another peculiar aspect of Frasassi caves is related to the presence of colonies of sulfur bacteria, and to the role that they play in the subterranean ecosystem [28-31]. These bacteria are chemolithoautotrophic for they fix carbon from atmospheric CO_2 to make organic compounds for their growth and gaining energy by coupling H_2S oxidation with O_2 or NO_3^- reduction. The constant and relatively abundant production of organic matter provides a dependable food source for a number of invertebrates that inhabit the sulfidic zones of the caves. These animals, including anellids, insects, gastropods, and crustaceans, are extremely specialized and have succeeded in colonizing the farthest explored reaches of the Frasassi caves. In these sulfidic parts of the cave system, fourteen species of invertebrates were classified, five of which are newly discovered taxa, and seven of which are endemic to the Frasassi cave [29].

An interesting side aspect of this biochemical activity is its possible relationship to the speleogenesis of the Frasassi complex. The natural inorganic oxidation of hydrogen sulfide is possible under normal atmospheric conditions but it is slow [32,33]. The bacterial activity, which is fundamental to the metabolism of these microorganisms, plays a central role in the acceleration of these reactions. It is, therefore, plausible that a large portion of the Frasassi caves was created, and is being created today, by the biologic actions of these sulfur-bacteria, which, as final product of their chemosynthetic metabolism, produce H_2SO_4, which then dissolves calcite in the cave system [31,34].

REFERENCES

[1] A. Montanari, S. Galdenzi, G. Rossetti, Drops of Time: strolling among rocks, field guide for the Frasassi Park with CD audio of Quaternary geophonic music, Osservatorio Geologico di Coldigioco (2002) 95 pp.

[2] B. Martinis, M. Pieri, Alcune notizie sulla formazione evaporitica del Triassico superiore nell'Italia centrale e meridionale, Mem. Soc. Geol. Ital. 4 (1964) 649-678.

[3] L. Anelli, M. Gorza, M. Pieri, M. Riva, Subsurface well data in the northern Apennines (Italy), Mem. Soc. Geol. Ital. 48 (1994) 461-471.

[4] P. Elter, G. Giglia, M. Tongiorgi, L. Trevisan, Tensional and compressional areas in the recent (Tortonian to present) evolution of the northern Apennines. Boll. Geofis. Teor. Appli, 17 (1975) 3-19.

[5] G. Lavecchia, G. Minelli, G. Pialli, Contractional and extensional tectonics along the cross-section Lake Trasimeno-Pesaro (Central Italy), in: The lithosphere in Italy: Advances in Earth Sciences Research, A. Boriani, M. Bonafede, G.B. Piccardo, G.B. Vai (Eds.), Accademia Nazionale dei Lincei, Roma, (1987) 177-94.

[6] D. Di Bucci, S. Mazzoli, Active tectonics of the Northern Apennines and Adria geodynamics: new data and a discussion. J. Geodyn. 42 (2002) 687-707.

[7] W. Spakman S. van der Lee, R. van der Hilst, Travel-time tomography of the European-Mediterranean mantle down to 1400 km. Phys.Earth Planet. Inter. 79 (1993) 3-74.

[8] N. D'Agostino, J.A. Jackson, F. Dramis, R. Funiciello, Interaction between mantle upwelling, drainage evolution, and active normal faulting: an example from the central Apennines (Italy), Geophys. J. Internat. 147 (2001) 475-484.

[9] A. Frepoli A., A. Amato, Contemporaneous extension and compression in the Northern Apennines from earthquake fault-plane solutions, Geophys. J. Internat. 129 (1997) 368-388.

[10] C. Chiarabba, L. Jovane, R. Di Stefano, A new view of Italian seismicity using 20 years of instrumental recordings. Tectonophysics 395 (2005) 251-268.

[11] P. Montone , M.T. Mariucci , S. Pondrelli, A. Amato, An improved stress map for Italy and surrounding regions (Central Mediterranean). J. Geophys. Res. 109 (2004) B10410, doi:

10.1029/2003JB002703.

[12] M. Battaglia, M.H. Murray, E. Serpelloni, E., R. Bürgmann, The Adriatic region: an independent microplate within the Africa-Eurasia collision zone, Geophys. Res. Lett. 31 (2004) L09605, 10.1029/2004GL019723.

[13] G. Lavecchia, F. Brozzetti, M. Barchi, J. Keller, M. Menichetti, Seismotectonic zoning in east-central Italy deduced from the analysis of Neogene to Present deformation and related stress fields, Geol. Soc. Am. Bull. 106 (1994) 1107-1120.

[14] G. Lavecchia, P. Boncio, N. Creati, A lithospheric-scale seismogenic thrust in Central Italy, J. Geodyn. 36 (2003) 79-94.

[15] M.R. Barchi, Lithological and structural controls on the seismogenesis of the Umbria region: observations from seismic reflection profiles, Boll. Soc. Geol. Ital. Volume Speciale 1 (2002) 855-864.

[16] P. Boncio, F. Brozzetti, G. Lavecchia, Architecture and seismotectonics of a regional low-angle normal fault zone in central Italy, Tectonics 19 (2000) 1038-1055.

[17] C. Collettini, M.R. Barchi, A low angle normal fault in the Umbria region (Central Italy): a mechanical model for the related microseismicity, Tectonophysics 359 (2002) 97-115.

[18] W. Alvarez, Drainage on evolving fold-thrust belts: a study of transverse canyons in the Apennines, Basin Research 11 (1999) 267-284.

[19] R. Mazzanti, L. Trevisan, Evoluzione della rete idrografica nell'Appennino centro-settentrionale, Geografia Fisica e Dinamica Quaternaria 1 (1978) 55-62.

[20] C. Cattuto, Correlazione tra piani carsici ipogei e terrazzo fluviali nella valle del Fiume Esino (Marche). Boll. Soc. Geol. Ital. 95 (1976) 313-326.

[21] A. Bocchini, M. Coltorti, Il complesso carsico Grotta del Fiume Grotta Grande del Vento e l'evoluzione geomorfologica della Gola di Frasassi: Mem. Ist. Ital. Speleo. 2 (1990) 155-180.

[22] S. Galdenzi, M. Menichetti, Occurrence of hypogenic caves in a karst region: Examples from Central Italy, Environ. Geol. 26 (1995) 39-47.

[23] L. Mayer , M. Menichetti, O. Nesci, D. Savelli, Morphotectonic approach to the drainage analysis in the North Marche region, central Italy. Quaternary International 102 (2003) 157-167.

[24] M.B. Goldhaber, W.L. Orr, Kinetic controls on thermochemical sulfate reduction as a source of sedimentary H_2S. Geochemical Transformations of Sedimentary Sulfur (ed. M. A. Vairavamurthy and M. A. A. Schoonen) Am. Chem. Soc. 612 (1995) 412-425.

[25] H.G. Machel, Bacterial and thermochemical sulfate reduction in diagenetic settings - old and new insights. Sed. Geol. 140 (2001) 143-175.

[26] A.A. Sicree H.L. Barnes, Upper Mississippi Valley district ore fluid model: The role of organic complexes. Ore Geol. Rev. 11 (1996), 105-131.

[27] S. Galdenzi, T. Maruoka, Gypsum deposits in the Frasassi caves, Central Italy: Journal of Cave and Karst Studies 65 (2003) 111-125.

[28] S. Galdenzi, M. Menichetti, S.M. Sarbu, A. Rossi, Frasassi caves: a biogenic hypogean karst? In: Proceedings of the European Conference Karst 99, P. Audora (Ed.), Université de Provance, Etudes de Géographie Physique 28 (1999) 101-106.

[29] S.M. Sarbu, S. Galdenzi, M. Menichetti, G. Gentile, Geology and biology of the Frasassi caves in Central Italy: an ecological multi-disciplinary study of a hypogenic underground karst system, in: Ecosystems of the World, D. Wilkens, D. Culver, W.F. Hamphreys (Eds.), Elsevier, New York, (2000) 359-378.

[30] A. Lyon, B. Koffman, K., Cleaveland, L., S. Mariani, S. Galdenzi, J. Macalady, Geomicrobiology of the Frasassi Caves, in: Frasassi 1989-2004: gli sviluppi nella ricerca, S. Galdenzi (Ed.), Consorzio Frasassi, Genga, (2004) 152-157.

[31] J.L. Macalady, E.H. Lyon, L.K. Albertson, B. Koffman, D.S. Jones, S. Mariani, S. Galdenzi, Dominant microbial populations in limestone-corroding stream biofilms, Frasassi cave system, Italy,

Appl. Environ. Microbio. (2006) 5596-5609.

[32] K. Chen, J.C. Morris, Kinetics of oxidation of aqueous sulfide by O2. Envir. Sci. Technol. 6 (1972) 529-537.

[33] J.Z. Zhang, F. J. Millero, Kinetics of Oxidation of Hydrogen-Sulfide in Natural-Waters. In Environmental Geochemistry of Sulfide Oxidation, C. N. Alpers and D. W. Blowes (Eds.), Washington, D.C. Am. Chem. Soc. (1994) 550 393-409.

[34] S. Galdenzi, S. Sarbu, Chemosintesi e speleogenesi in un ecosistema ipogeo: i rami sulfurei delle Grotte di Frasassi (Italia Centrale). Le Grotte d'Italia 1 (2000) 3-18.

Notes

Microbial life along environmental gradients in karst habitats

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Compared to other habitats, one of the advantages of ecological studies in karst and caves is that it is rather easy to study organisms and their interactions along physicochemical gradients, e.g. oxygen, temperature, relative humidity, and light irradiation. Summarized here are three of our projects to examine the effects of environmental gradients on community composition and structure in karst settings, and the effects of the community on changing the gradients over time.

(i) To evaluate the nature and behaviour of ecological communities along sulphide and oxygen gradients, the sulphidic karst spring Žveplenica in Dolenja Trebuša (Slovenia) was selected. The spring has constant physicochemical parameters, which have been monitored over one year. Evaluation of total biodiversity using 16S rDNA (Archaea, Bacteria) and 18S rDNA (Eukarya) was done for the sulphidic spring at the orifice of the spring, with 34 μmol L^{-1} dissolved sulphide and <0.1 μmol L^{-1} dissolved O_2, and 0.2 m downstream where the microbial mat is in water with >190 μmol L^{-1} dissolved O_2 and <10 μmol L^{-1} dissolved sulphide. The archaeal communities at the two sites were statistically similar ($P = 0.372$), with 85% of the gene sequences being shared, but the bacterial communities were not similar ($P < 0.0001$). Among the Archaea, sequences were affiliated with methanogens, halophiles, and two large unclassified groups previously identified from hypersaline microbial mats from Guerrero Negro, Baja California, and microbial mats from Koz Spring in Yellowstone National Park, Wyoming. The bacterial diversity from the mats was dominated by *Gammaproteobacteria*, and specifically groups affiliated with *Thiothrix* spp. Other sequences were affiliated with the *Deltaproteobacteria*, *Betaproteobacteria*, *Alphaproteobacteria*, *Firmicutes*, the SR1 and OP4 Candidate Divisions, *Planctomycetes*, *Deferribacteres*, and *Bacteroidetes*. The eukaryotic diversity from the downstream mat was more diverse than the orifice sample. Interestingly, both communities contained zoopagalean fungi. Molecular data on meiofauna was supplemented with the microscopic analysis. Cyclopoida and Harpacticoida copepods were the most abundant groups recovered. Although copepods colonize cave and subterranean habitats, their diversity and role in this sulphidic system is not well understood. As Žveplenica has a very short watercourse, to examine phototrophic community from a sulphidic spring downstream, another site in the same karst area was selected; an artificial well in the Sovra valley (Slovenia). Microbial white filaments originating from the spring were intertwined with green mats composed mostly of green alga *Stigeoclonium* (Chlorophyta), and *Anabaena* (Cyanobacteria), and *Epithemia* (Chrysophyta). Phototrophic community composition downstream gradually adopted the structure of the neighbor stream Sovra.

(ii) Natural cave entrances represent an ideal laboratory to study the ecology of phototrophic organisms. At the cave temperature (9°C) and low light irradiation (< 10 μmol photons m^{-2} s^{-1}), cyanobacteria and green algae favour synthesis of accessory photosynthetic pigments. The most important factor for algal distribution with a photon flux of 2 μmol photons m^{-2} s^{-1} was temperature in a cave entrance ($P<0.05$). We identified that phototrophs in cave environments have slower growing rates and must deal with the presence of predatory organisms. Specifically, in complex phototrophic mats from cave entrances, protozoa and free-living amoebae, which contribute to nutrient cycling and phototrophs succession, have been frequently identified. From a stromatolitic stalagmite phototrophic mat from the entrance of Škocjanske jame (Slovenia), a new vahlkampfiid amoeba, *Allovahlkampfia spelaea* gen. nov., sp. nov., has been recently described.

(iii) Climatic conditions in caves are relatively stable, but they can vary depending on the number of entrances and passage or fracture connection with the surface. Several cave niches with distinct atmospheric parameters (e.g. Radon, CO_2) can be distinguished where concentrations of airborne organisms can vary. Results from the largest Slovenian show cave, Postojnska jama, indicated no correlation between microbial - fungal and bacterial - cfu and temperature, relative humidity, air current, air pressure and dust concentration. Only a negative correlation (-0.63) between fungal cfu and CO_2 ($P<0.05$) was observed. Air currents and river flow into the cave introduced more fungi than bacteria, 23Xand 11X more, respectively. Lower ratios of fungi vs. bacteria were observed in the tourist-visted sections of the cave (annually 500,000 visitors); the highest absolute number of 137 bacterial cfu m^{-3} was obtained. Interestingly, in the passage with elevated Ra concentrations, the ratio of fungi vs. bacteria was low (0.7) and was represented by only 9 cfu m^{-3}. The highest fungal cfu was calculated in the vicinity of tourist underground train (738 cfu m^{-3}). The results provide estimates of potential inflow of biological particles into a cave, their local distribution, and influence of visitors in the tourist parts of a cave.

Notes

Notes

Microbial food webs in Movile Cave, Romania

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Microbial diversity in Movile Cave (Romania) was studied using *Bacterial* and *Archaeal* 16S rRNA gene sequence and functional gene analyses, including ribulose 1, 5 bisphosphate carboxylase / oxygenase (RuBisCO), *soxB* (sulfate thioesterase/ thiohydrolase) and *amoA* (ammonia monooxygenase). Sulfur oxidizers from both *Gammaproteobacteria* and *Betaproteobacteria* were detected in 16S rRNA, *soxB* and RuBisCO gene libraries. DNA-based stable isotope probing (SIP) analyses using ^{13}C-bicarbonate demonstrated that *Thiobacillus* spp. were most active in assimilating CO_2 and also implied that ammonia and nitrite oxidizers were active during incubations.

SIP experiments with $^{13}CH_4$ revealed the presence of active methanotrophs. Also sequences from extant methylotrophs indicating that cycling of one-carbon compounds is an important process within Movile Cave. *Archaeal* 16S rRNA sequences retrieved were restricted to two groups, namely the Deep-sea Hydrothermal Vent Euryarchaeota group and the Miscellaneous Crenarchaeota group.

No sequences related to known sulfur-oxidizing *Archaea*, ammonia-oxidizing *Archaea*, methanogens or anaerobic methane-oxidizing *Archaea* were detected in this clone library. The results provide molecular biological evidence to support the hypothesis that life in Movile Cave is driven by chemolithoautotrophy, mainly through sulfur oxidation by sulfur-oxidizing *Bacteria,* and methanotrophy and reveal that ammonia - and nitrite-oxidizing *Bacteria* may also be major primary producers in Movile Cave.

Notes

Reconnaissance of ostracod assemblages in the Frasassi cave system, Sentino River, and adjacent sulfidic spring

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A total of 38 sediment samples systematically taken near solstices and equinoxes from spring 2005 to summer 2008, were collected from six phreatic sulfidic lakes of the Frasassi cave system (Grotta del Fiume-Grotta Grande del Vento), at depths varying from 1 cm to a maximum of 1 m below water surface, the sandy shore of the Sentino River, and a shallow puddle at a sulfidic spring discharging from the cave into the river immediately outside the cave. In addition to these, we collected and analyzed 15 samples of subfossil eel remains resting up to 4 m above present water table on the shores of Lago delle Anguille, in the inner part of the Grotta del Fiume. This collection yielded a total 5,585 ostracod valves representing 23 different species, as reported in Table 1, and shown in Figure 1.

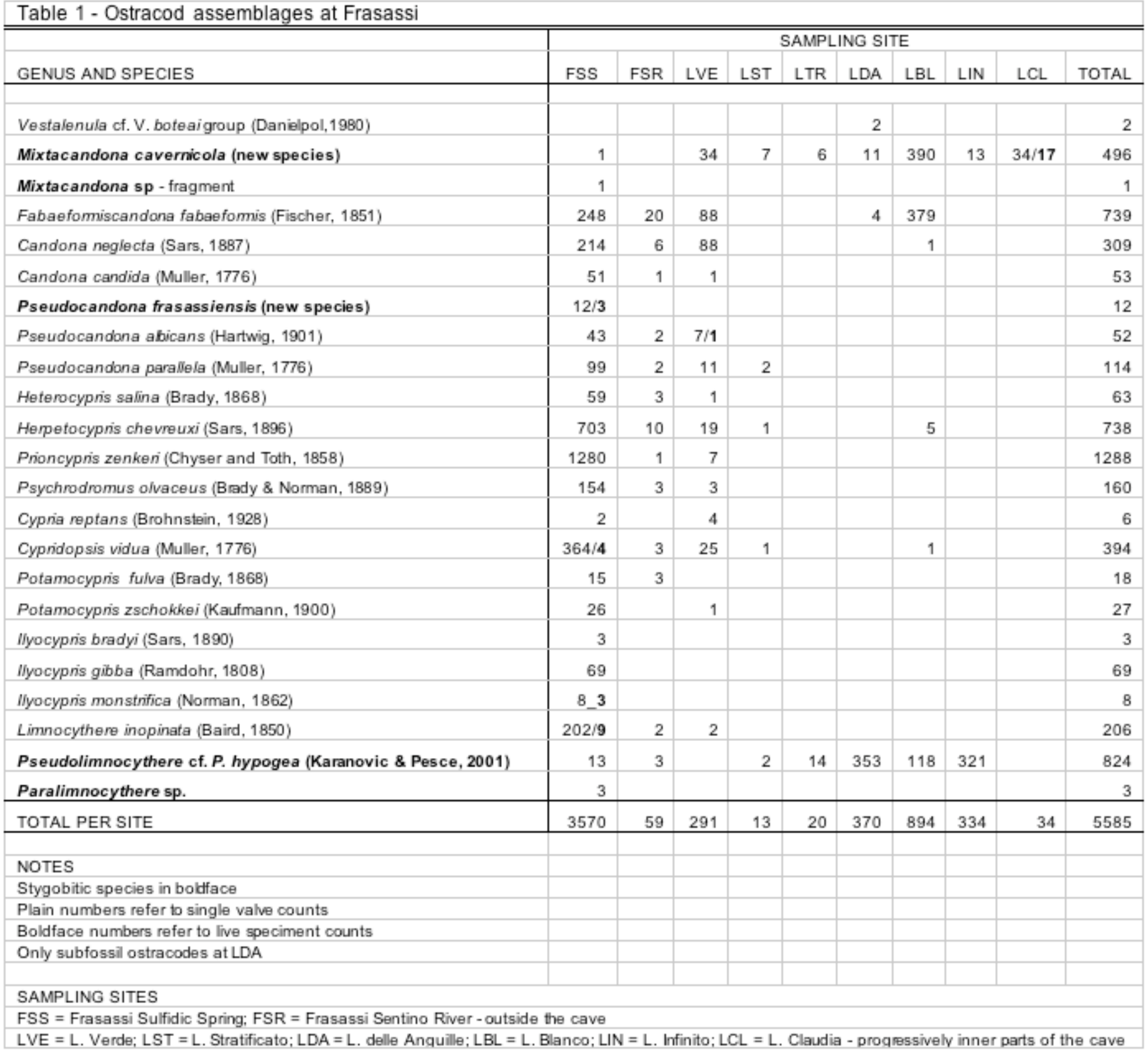

Table 1 - Ostracod assemblages at Frasassi

	SAMPLING SITE									
GENUS AND SPECIES	FSS	FSR	LVE	LST	LTR	LDA	LBL	LIN	LCL	TOTAL
Vestalenula cf. V. *boteai* group (Danielpol,1980)						2				2
***Mixtacandona cavernicola* (new species)**	1		34	7	6	11	390	13	34/**17**	496
***Mixtacandona* sp** - fragment	1									1
Fabaeformiscandona fabaeformis (Fischer, 1851)	248	20	88			4	379			739
Candona neglecta (Sars, 1887)	214	6	88				1			309
Candona candida (Muller, 1776)	51	1	1							53
***Pseudocandona frasassiensis* (new species)**	12/**3**									12
Pseudocandona albicans (Hartwig, 1901)	43	2	7/**1**							52
Pseudocandona parallela (Muller, 1776)	99	2	11	2						114
Heterocypris salina (Brady, 1868)	59	3	1							63
Herpetocypris chevreuxi (Sars, 1896)	703	10	19	1			5			738
Prioncypris zenkeri (Chyser and Toth, 1858)	1280	1	7							1288
Psychrodromus olvaceus (Brady & Norman, 1889)	154	3	3							160
Cypria reptans (Brohnstein, 1928)	2		4							6
Cypridopsis vidua (Muller, 1776)	364/**4**	3	25	1			1			394
Potamocypris fulva (Brady, 1868)	15	3								18
Potamocypris zschokkei (Kaufmann, 1900)	26		1							27
Ilyocypris bradyi (Sars, 1890)	3									3
Ilyocypris gibba (Ramdohr, 1808)	69									69
Ilyocypris monstrifica (Norman, 1862)	8_**3**									8
Limnocythere inopinata (Baird, 1850)	202/**9**	2	2							206
***Pseudolimnocythere* cf. *P. hypogea* (Karanovic & Pesce, 2001)**	13	3		2	14	353	118	321		824
***Paralimnocythere* sp.**	3									3
TOTAL PER SITE	3570	59	291	13	20	370	894	334	34	5585

NOTES
Stygobitic species in boldface
Plain numbers refer to single valve counts
Boldface numbers refer to live speciment counts
Only subfossil ostracodes at LDA

SAMPLING SITES
FSS = Frasassi Sulfidic Spring; FSR = Frasassi Sentino River - outside the cave
LVE = L. Verde; LST = L. Stratificato; LDA = L. delle Anguille; LBL = L. Blanco; LIN = L. Infinito; LCL = L. Claudia - progressively inner parts of the cave

Eighteen of these are epigean species, which are common in fresh waters in Italy and throughout Europe, but some of them were identified also in the Frasassi cave. Of the five stygobitic species found both outside and inside the cave, two are new species, *Mixtacandona cavernicola* and *Pseudocandona frasassiensis*, the former found only in cave samples, and the latter only in sulfidic spring sediment outside the cave.

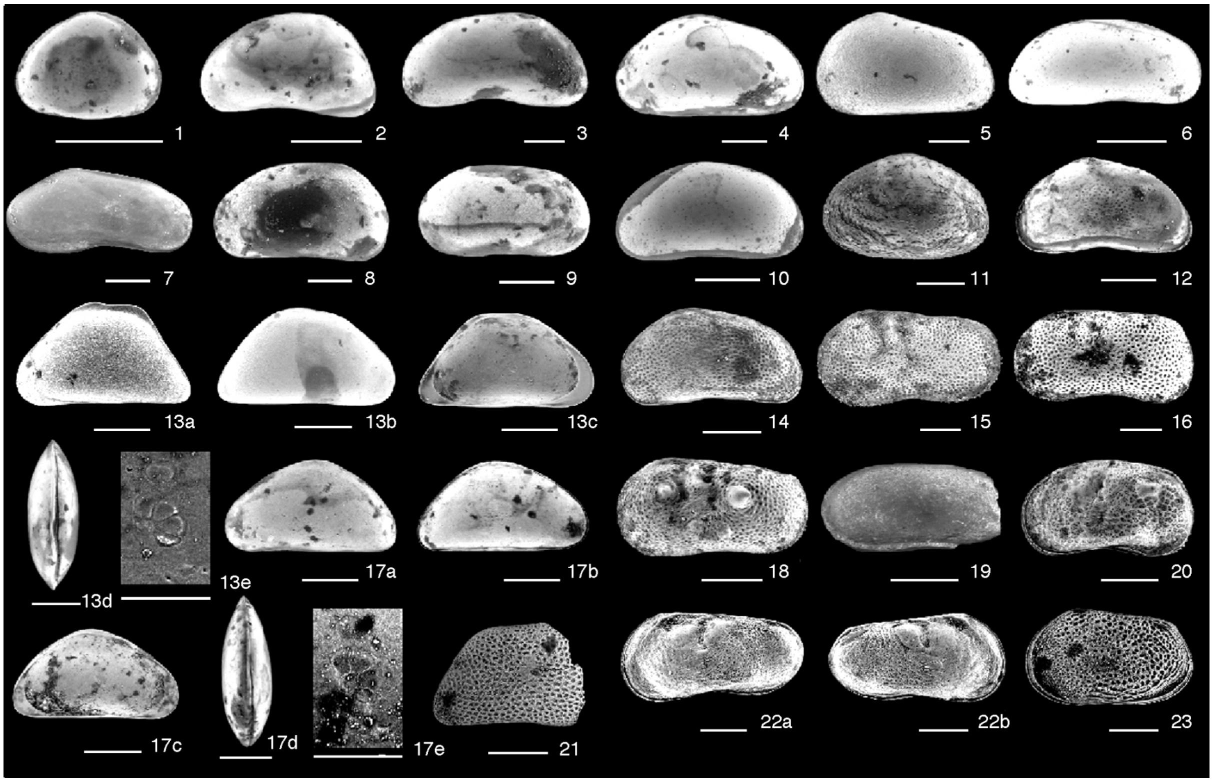

Fig. 1 - Ostracod species at Frasassi; scale bar = 100 µm; lv = left valve; rv right valve; elv = external lateral view1. *Cypria reptans* (Bronshtein, 1928), lv, elv. 2. *Candona candida* (Müller, 1776),), lv, elv; 3. *Candona neglecta* (G. O. Sars, 1887)), lv, elv; 4. *Heterocypris salina* (Brady, 1868), lv, elv; 5. *Prionocypris zenkeri* (Chyser & Toth, 1858), lv, elv; 6. *Herpetocypris chevreuxi* rv, elv.; 7. *Fabaeformiscandona fabaeformis* (Fischer, 1851), rv, elv. 8. *Pseudocandona albicans* (Brady, 1864), lv, elv; 9. *Pseudocandona parallela* (G. W. Müller, 1900), lv, elv; 10. *Psychrodromus olivaceus* (Brady and Norman, 1889), lv, elv; 11. *Cypridopsis vidua* (O. F. Müller, 1776), lv, elv; 12. *Potamocypris fulva* (Brady, 1868),rv, elv.13a. *Mixtacandona cavernicola*, lv, elv. female, elv. 13b. *Mixtacandona cavernicola*, rv female, elv; 13c. *Mixtacandona cavernicola*, lv, elv; 13d. *Mixtacandona cavernicola*, dorsal view; 13e. *Mixtacandona cavernicola*, abductor muscle scars.; 14. *Potamocypris zschokkei* (Kaufmann, 1900), rv, elv; 15. *Ilyocypris gibba* (Ramdohr, 1808), lv, elv; 16. *Ilyocypris bradyi* (Sars, 1890), rv, elv; 17a. *Pseudocandona frasassiensis*, lv, elv; 17b *Pseudocandona frasassiensis*, rv, elv; 17c. *Pseudocandona frasassiensis*, lv, internal view; 17d. *Pseudocandona frasassiensis*, dorsal view; 17e. *Pseudocandona frasassiensis*, abductor muscle scars; 18. *Ilyocypris monstrifica* (Norman, 1962), lv, elv; 19. *Vestalenula* cf. V. *boteai* (Danielpol, 1970), rv, elv; 20 *Limnocythere inopinata* (Baird, 1850), lvv, elv; 21; fragment of *Mixtacandona* sp., lv, elv; 22a *Paralimnocythere* sp., lv., elv.; 22b *Paralimnocythere* sp., rv., elv. 23. *Pseudolimnocythere hypogea* (Klie, 1938), rv, elv.

Despite the large number of ostracod valves counted and identified at a specific level, only a few specimens were found live. Among them, *Pseudocandona frasassiensis*, *Limnocythere inopinata*, *Ilyiocypris monstrifica*, and *Cypridopsis vidua* were found in the sulfidic spring outside the cave, while one live specimen of *Pseudocandona albicans* found at Lago Verde, and 17 specimens of *Mixtacandona cavernicola* from Lago Claudia, in the innermost part of the Grotta Grande del Vento, are the only species recovered live thus far from the cave. *M. cavernicola* is the dominant species at Lago Blanco and in other lakes in the inner part of Grotta del Fiume. Eleven valves of this species were also recovered from the remains of subfossil eels as old as 7,300 years, suggesting that *M. cavernicola* has been present in the cave for a long period of time.

The paucity of live specimens in these environments, which is in marked contrast with the large number of ostracod valves recovered in cave samples and the sulfidic spring outside the cave, severely limits the interpretation of the ecology of these organisms, their endemism, and their stationarity and role in the sulfidic, autotrophic ecosystem of the cave.

The ostracod assemblage at Lago Verde, the sulfidic pool of the Grotta del Fiume closest to the natural entrance of the cave, is represented by 13 epigean species. Of a total of 291 valves identified in several samples recovered from this sulfidic lake, only one live specimen of *Pseudocandona albicans* was found. A large number of these dead valves are believed to have washed into Lago Verde when, perhaps in the recent past, the Sentino River was flowing at a higher level than today.

Numerous valves of the stygobitic species *Pseudolimnocythere* cf. *P. hypogea* (Klie), were found in the inner lakes of the Grotta del Fiume, as well as in the remains of the subfossil eels at Lago delle Anguille. Rare valves of this species were also identified in the sulfidic spring and Sentino River sediments, outside the cave. The lack of live specimens suggests that we have yet to locate the areas in the phreatic lakes inhabited by this species. Relatively abundant valves of *Fabaeformiscandona fabaeformis* were found in the sulfidic spring, at Lago Verde, and Lago Blanco, suggesting that this epigean species can adapt to living in the subterranean environment of the cave. Once again, however, we are frustrated in our efforts to recover live material.

Variability of the environmental conditions in the sampled sites, both inside and outside the cave, primarily due to seasonal changes in rain fall and consequent variations of the phreatic water table level and sulfide concentration, further complicates the study and even the sampling of the ostracod assemblages. Improvement of sampling techniques (i.e. the consistent use of a filtered pump) and a correct choice of sampling times (i.e. from late spring to early fall) will hopefully yield more live specimens from our sample stations, especially in the sulfidic spring, Lago Verde, and Lago Claudia, which revealed the presence of live specimens. Careful analysis of shell morphometrics, study of soft anatomy, comparison to other described species, combined in the future with genetic analysis will hopefully lead to a better understanding of the phylogenesis of these ostracodes and the role they play in the sulfidic ecosystem of Frasassi.

Notes

S isotope investigation of sulfur cycling in the Frassassi cave system: A case study of chemotrophic sulfide oxidation

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S isotope values of sulfur species in natural systems reflect the biological and geochemical processes that contribute to the sulfur cycle in these systems. These processes each produce a well-documented range of fractionations in the major isotopes of sulfur (^{34}S and ^{32}S presented as $\delta^{34}S$, the ratio of heavy to light isotopes in a sample relative to a standard). As a result, the sedimentary $\delta^{34}S$ record has been used to infer both the timing of evolution of microbial S metabolisms and the timing and structure of the oxygenation of the earth's atmosphere. Minor isotopes of sulfur (^{33}S and ^{36}S) are subject to similar inorganic and organic fractionation mechanisms. In addition, the redistribution of all four S isotopes within biogeochemical systems (at both the cellular and ecosystem level) produces unique patterns that can be used to assess the contribution of different pathways (enzymatic or biogeochemical) to the measured isotopic values.

The majority of the isotopic signal in modern systems is due to the reduction of sulfate to sulfide by dissimilatory sulfate-reducing prokaryotes and the biological disproportionation of intermediate sulfur compounds to sulfate and sulfide. Sulfide oxidation processes are also important in some environments, and can occur through a variety of pathways, both biological (phototrophic and chemotrophic) and abiotic (e.g., with O_2). Each of these sulfide oxidation pathways has been shown to produce small, but distinct, isotope effects in $\delta^{34}S$ (Figure 1, shown for the reactant sulfide).

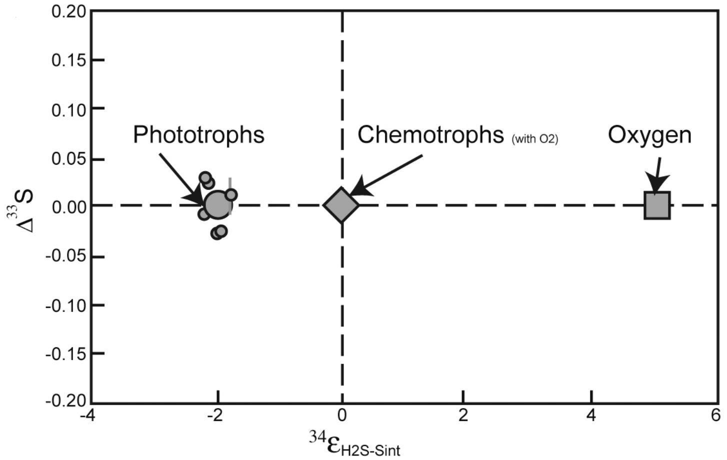

Fig. 1

Ecosystem-scale S isotope models suggest that these small isotope effects can significantly alter the isotopic composition of sulfur species in natural systems (shown in Figure 2 as sulfate) if sufficient oxidation is occurring (Zerkle et al., 2009).

Previous experimental studies have measured negligible isotope effects in $\delta^{34}S$ during biological (chemotrophic) S-oxidation with O_2 (Fry et al., 1984). Larger fractionations have been observed when minor reaction products, such as polythionates, are formed (Kaplan and Rittenberg, 1964), which could be a significant process in natural systems. We have measured larger fractionations in initial experiments with the chemotrophic S-oxidizer *T. nivea* as well (unpublished data).

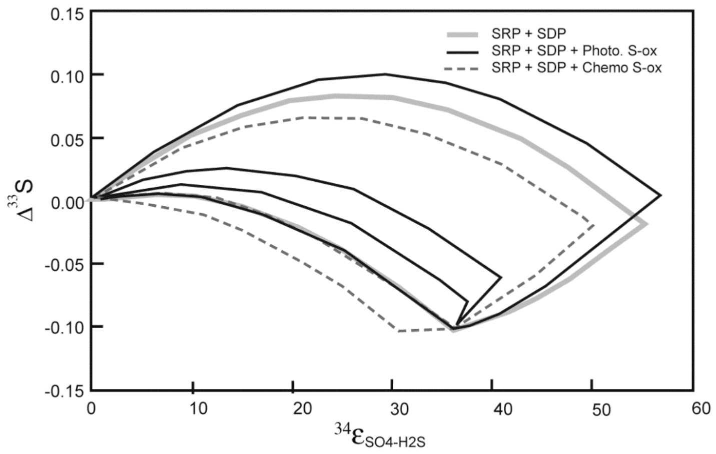

Fig. 2

Direct examination of isotope effects during sulfide oxidation in natural systems has been difficult due to the rapid recycling of S-intermediate reaction products, and the dominance of other sulfur metabolisms in most of the environments studied. The Frassassi cave system is the ideal environment in which to investigate sulfur isotope effects in a sulfur ecosystem dominated by chemotrophic S-oxidation processes. The wide range of sulfide and oxygen concentrations in the cave waters and the well-documented associations of microbial biofilms (Macalady et al., 2008) will provide the context to quantify the effects of these environmental and biological parameters on isotopic fractionation at a local scale. Additionally, trends in the occurrence of sulfur metabolisms and the resulting isotopic values across the ecosystem as a whole will provide clues to the type of isotopic patterns we might expect from similar oxygen-limited systems preserved in the geologic record.

REFERENCES

Fry, B., Gest, H., and Hayes, J. M., 1984. Isotope effects associated with the anaerobic oxidation of sulfide by the purple photosynthetic bacterium, *Chromatium vinosum*. *FEMS Microbiology Letters* **22**, 283-287.

Kaplan, I. R. and Rittenberg, S. C., 1964. Microbiological fractionation of sulphur isotopes. *Journal of General Microbiology* **34**, 195-212.

Macalady, J. L., Dattagupta, S., Schaperdoth, I., Jones, D. S., Druschel, G. K., and Eastman, D., 2008. Niche differentiation among sulfur-oxidizing bacterial populations in cave waters. *The ISME Journal*, 1-12.

Zerkle, A. L., Farquhar, J., Johnston, D. T., Cox, R. P., and Canfield, D. E., 2009. Fractionation of multiple sulfur isotopes during phototrophic oxidation of sulfide and elemental sulfur by a green sulfur bacterium. *Geochimica Et Cosmochimica Acta* **73**, 291-306.

Notes