underwater TECHNOLOGY

The underwater exploration of the Merro sinkhole and the associated diving physiological and psychological effects

Roberto Palozzi
Department of Biology, University of Rome "Tor Vergata", Italy

Giorgio Caramanna
Centre for Innovation in Carbon Capture and Storage (CICCS), Fuels and Power Technology Research Division Faculty of Engineering, University of Nottingham, UK

Patrizia Albertano, Roberta Congestri, Laura Bruno and Antonio Romano
Department of Biology, University of Rome "Tor Vergata", Italy

Maria Gabriella Giganti
Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Italy

Rossella Zenobi
Department of Internal Medicine, University of Rome "Tor Vergata", Italy

Corrado Costanzo and Giuliana Valente
Centro Iperbarico Romano, Italy

Diego Polani
Department of Medicine, University of Florence, Italy

Mario Vecchio and Maria Vinci
Environment Service, Provincia di Roma, Italy

Valerio Sbordoni
Department of Biology, University of Rome "Tor Vergata", Italy

Abstract

The Pozzo del Merro (Merro sinkhole), a few kilometres away from Rome, Italy, is the deepest flooded karstic cavity known in the world. Over the last two years, a multi-disciplinary scientific research project studied the almost unknown aquatic ecosystem of the sinkhole while also studying the psychological and physiological reactions of scientific divers operating in the very hostile underwater environment. This paper presents a preliminary overview of the seven studies carried out in parallel and attempts to highlight the fundamental role of scientific diving in contributing to increased knowledge about this extreme environment. The discovery of two exotic species in the sinkhole represents a paradigmatic case of the problem of invasive species introduction in such a unique environment. The project also included research on human diving physiology, pathology, and psychology through monitoring of all the divers (plus one free-diver) working in the Merro sinkhole.

Keywords: sinkhole, cave diving, fern, newt, amphipod, invasive species, SCUBA, diving physiology, diving psychology, free-diving

1. Introduction

The Merro Scientific Project (MSP) is a biennial research project whose aim is to study the ecosystem of the submerged part of the Pozzo del Merro (the Merro sinkhole, which is a karstic doline), and some physiological and psychological reactions of the divers performing the scientific samplings. The unique approach of the MSP has been the multi-disciplinary approach that has allowed it to cater to the needs of seven different scientific disciplines by means of scientific diving.

The present account provides a preliminary overview (as providing complete data from seven different scientific works is outside the scope of this paper) of the research carried out in the framework of the MSP, with a focus on the role of the scientific diving.

2. Study site

Presently, Pozzo del Merro is the deepest sink-hole known in the world (Gary et al., 2003). It is located within the territory of Sant'Angelo Romano (a small village about 30km outside of Rome) at 140m

Fig 1: The lake inside the Merro sinkhole completely covered by *Salvinia molesta* (courtesy of Roberto Palozzi©)

altitude in the southern slopes of the Cornicolani mountains (4 2′02′14; 12″35′52) (Giardini et al., 2001). It is the largest and most impressive demonstration of karstification in this area (Segre, 1947, 1948; Maxia, 1954; Casale et al., 1963; Caramanna, 2002). The outcrop consists of limestone of Liassic age (Chiocchini and Mancinelli, 1978) and the area is characterised by three regional fault systems with directions: northwest–southeast (NW–SE), northeast–southwest (NE–SW) and north–south (N–S) (Mattei et al., 1986). The presence of these faults enhanced the karst erosion on the bedrock with formation of several erosive structures, such as sinkholes and caves.

The Merro sinkhole has a funnel-like shape with a diameter of about 160m at ground level, narrowing to 25m at about 70m depth. At the 70m depth the well is flooded and this sector is characterised by a sub-vertical conduit with several secondary cavities along the walls. Karst erosion is very strong along the whole structure, with formation of thin blades of limestone.

The existence of the sinkhole has been well known for hundreds of years and it has been considered a tourist attraction since the nineteenth century (e.g. Abbate, 1890). The first scientific information on the sinkhole dates back to 1886 (Tuccimei, 1886) even if the first real scientific research started after World War II (Segre, 1947, 1948). For the next five decades studies focused mainly on the geological and morphological aspects of the doline emerged part, while very little – if any – attention was dedicated to the underwater structure of the lake or the biotic community of the whole well. In 1975, the first bathymetric survey was attempted by an electronic echo-sounder and the depth of the lake was erroneously indicated as 70m. In 1999, divers explored the sinkhole for the first time, demonstrating that the bottom was much deeper than 100m (Caramanna, 2002). In 2002, after three explorative underwater campaigns led by remote operated vehicles (ROV) (Caramanna, 2001), a fire department ROV reached the depth of 392m from the water surface (which could represent the bottom of the well) and is the highest value ever recorded for a sinkhole (Caramanna, 2002; Gary et al., 2003). The total well length from the ground level is about 460m.

In 2006, there was a growing demand for a thorough study of the underwater ecosystem of the Merro sinkhole and its relationship with changing abiotic conditions. In 2003, there was unexpected and inexplicable invasion of the aquatic, Brazilian tropical fern, *Salvinia molesta* D.S. Mitchell (Giardini, 2003, 2004). Within a few months, the fern smothered the lake surface replacing the endemic *Lemna minor* L., creating a plug more than 5cm thick on which terrestrial vegetation started to grow, as shown in Figure 1. The ecological consequences of this rampant invasion were unknown, and they raised several questions, such as the survival of the populations of two newt species, the *Triturus carnifex* (Laurenti) and *Lissotriton vulgaris* L.; an endemic species of amphipod, *Niphargus cornicolanus* Iannilli–Vigna Taglianti, recently discovered in the sinkhole (Iannilli and Vigna Taglianti, 2005); and the status of the almost completely unknown community of microalgae and cyanobacteria colonising the sinkhole submerged walls.

Although protection laws have been issued since 1970, the *S. molesta* invasion presented a problem of effective preservation of the sinkhole that only in

1997 had became part of a protected area, thanks to the creation of the natural reserve of *Macchia di Gattaceca e Macchia del Barco*, whose management was entrusted to the Province of Rome (Law of Latium Region n. 29/97). Since the creation of the protected area, the Province of Rome initiated activities aimed at the preservation and study of this geographical site. This was achieved in collaboration with research institutions, the fire department, and state forestry department. Most attempt to prevent disturbances from various origins (e.g. slope stability, abandonment waste, alien species, abuses related to residential development) that could compromise the delicate equilibrium of the sinkhole (European Community Commission, 2008).

3. Merro scientific project

A research campaign to study the underwater biotic community and its linkages with the abiotic structure began in April 2007 and was based on underwater observations and samplings led by professional cave-divers and researchers. The environmental conditions made underwater activities in the Merro sinkhole difficult principally because the height difference between the bottom of the well and the water surface could only be negotiated through use of ropes and mountaineering techniques. Because of these difficulties, MSP divers aimed to gather as much information for as many different research fields as possible and optimised the diving by collecting samples for use in studies of the hydrogeology, microbiology, zoology and archaeology of the area during the same diving programme.

Between April and May 2007 the team of divers lived and worked permanently in the Merro sinkhole carrying out tens of scientific dives. Since then further control dives have been repeated seasonally, until October 2009 when the MSP ended. The MSP provided a unique opportunity to study the SCUBA divers' reaction (between them and also of a free-diver) to being exposed to the stress of such a claustrophobic environment. Therefore, the project also included research on human diving physiology, pathology, and psychology through continuous monitoring of all the divers working in the Merro sinkhole. Spirometry and oxygen saturation were employed to assess the divers' physiological changes before and after immersion. In addition, there were assessments of instrumentation reliability; and operational efficiency of the medical and technical staff in a hostile environment with limited space to operate. Levels of some hormonal parameters which may be expected to change during stressful events, both of physical and mental nature, were also measured based on the proposed interaction between activation of the hypothalamus with related metabolic changes involving the nervous, endocrine and immune systems (Chrousos, 1995).

On the basis of scientific feedback, an attempt to restore the original habitat conditions was made in March 2009 involving the removal and the eradication of invasive exotic plants. This had the potential of being a very difficult task because of the hydro-geological peculiarities of the sinkhole. These operations were carried out with the support of the Rome fire department's SCUBA divers.

4. Diving operations and safety

The extreme steepness of the doline walls meant that all the gear had to be carried manually to the water surface, requiring mountaineering techniques. An Italian Red Cross (IRC) team set up a fixed rope system on which the operators attached themselves. A small wood platform was built inside the well and positioned on the water surface to allow divers and support staff to operate. Since it was not possible to carry the single tanks (15lt) and double tanks (12 + 12lt., 250bar) up and down every day, a small petrol engine compressor was placed close to the water surface to refill them.

4.1. Diving

Altogether 12 divers (11 SCUBA divers and 1 free-diver) participated in the underwater operations. At the most four divers were allowed to be in the water at any one time, although there were usually no more than two or three. The choice of compressed air as the only type of breathing gas was dictated by the logistical problems of moving equipment down to the diving station. This constraint limited underwater operations to 50m maximum depth; no dives exceeding decompression limits (except few exploratory dives led by only two divers); and only one dive per day if deeper than 20m. The 50m limitation was established because nitrogen narcosis becomes a factor of considerable risk even for very experienced and well trained diver below 55–60m (Mount and Gilliam, 1993; Schwerzmann and Seiler, 2001; Egstrom, 2006; Southerland, 2006; Lang, 2009). At this depth the oxygen partial pressure is below the recommended threshold of 1.4/1.3atm for technical divers engaged in demanding activities (Schwerzmann and Seiler, 2001; Brubakk and Gutvik, 2006).

Multiple dives deeper than 20m were not allowed in order to avoid unacceptable levels of physical and psychological stress. At the depth of about 25m the sinkhole became a narrow pipe with a sub-vertical path which presented very challenging and demanding diving conditions. All the dives carried out deeper than 20m were conducted using cave-diving techniques (Balcombe et al., 1990; Farr, 2003; Casati, 2007).

4.2. Safety

To guarantee the operator safety, two IRC teams (experts on mountain rescue) prepared a system to recover an injured person inside the well and monitored the scientific activities. Two ambulances were present on the top of the sinkhole and one helicopter was always on alert and ready to intervene if necessary. The underwater programmes were designed in cooperation with the Roman Hyperbaric Center (RHC), to ensure maximum protection for the divers. A hyperbaric doctor from RHC continuously monitored the divers and was present on the platform on the water during the dives; they were the only person authorised to take human biological samples.

In case of a DCS incident, the on-site doctor would coordinate the recovery from the water surface to the decompression chamber at the RHC following Kindwall's protocol (Kindwall and Whelan, 1999). It identifies four stages of the management and transportation of an injured diver in a particularly hostile environment:

- prompt emergency intervention;
- probable diagnosis;
- transportation to hyperbaric centre; and
- treatment in hyperbaric chamber.

Since it is essential in cases of DCS to reduce the response time between the occurrence of an accident and an effective first aid treatment, all the supposed initial symptoms of a potential DCS (such as unwarranted sense of fatigue, malaise, itching and/or tingling, vague pains, numbness and weakness in the limbs) were continuously checked (Davis and Elliott, 1982; Francis et al., 1989). This was to ensure a well-timed medical treatment at the accident site with the diagnostic evaluation, administration of normobaric 100 per cent oxygen and preparation for transportation (Moon, 1996).

5. Fields of research

5.1. Hydrogeology

Water samples were collected by SCUBA divers using plastic bottles filled with de-ionized water, which was purged once at the level to be sampled using the spare regulator air and filled with the water sample. The procedure allowed the collection of the sample with the minimum disturbance.

Chemical analyses were performed in the field (pH, total dissolved solids and bicarbonate) and in the lab (main ion concentration) by means of liquid chromathography. The temperature was measured by an electronic thermometer data-logger with a resolution of 0.1°C.

The preliminary results of the analyses and the observations conducted during MSP suggested the water body was chemically stable but that the water level was falling. Water levels had decreased by about 7.5–8m in 10 years of which 4.5–5m in the last 3 years (2007–2009; values refer to a bar positioned in 1998 to indicate the water surface at that time). The drop could be related to a general lowering of the regional aquifer (Caramanna, 2002) although over-pumping activity in some travertine quarries a few kilometres south of the sinkhole may also have had an influence.

The water chemistry is bicarbonate-calcic. Samples collected along the water column highlighted a progressive reduction of pH from neutrality up to a minimum value of 6.57 at about 100m. Over the same distance there was an increase in conductivity (up to 1.3ms/cm) from the water surface. As in a previous study, an anomalous concentration in sulphur was measured in some of the deepest samples (Caramanna, 2002); the water temperature (15.9°C) was constant all along the sinkhole.

5.2. Microbiology

Micro algae and cyanobacteria colonising the sub-vertical limestone walls of the Merro sinkhole were collected on three occasions: two times (summer and winter) while the water surface was covered by *S. molesta* and one (summer) after the Brazilian fern removal and the re-colonisation by *L. minor.* Divers were asked to try to identify spots that differed in colour from the uniform brown shade of the walls. The almost complete darkness of the environment meant the inspection required commercial underwater lights, and the divers had to be cautious not to lift the slight organic suspension adhering to the rocks. When a lit section appeared to be more reddish or yellowish in tint, the bio-films (together with the very brittle, superficial layer of limestone) were scraped off using a small, sharp underwater knife.

Bio-films were collected at two depths (5 and 8m) during the first and second sampling events, and at three depths (5, 8 and 11m) in the last sampling. There was no absolute correspondence between the depth values of the different sampling events because of the marked water level variation.

In the laboratory, aliquots of samples were fixed in 2% formaldehyde for microscopy analysis, and others put in 25mL culture flasks with BG-11 medium, and exposed to room temperature and light for further studies on cultures; some fresh material was immediately observed using light microscopy (Zeiss Axioskop at 40x magnification). An acidic stain, specific to the mucopolysaccharides

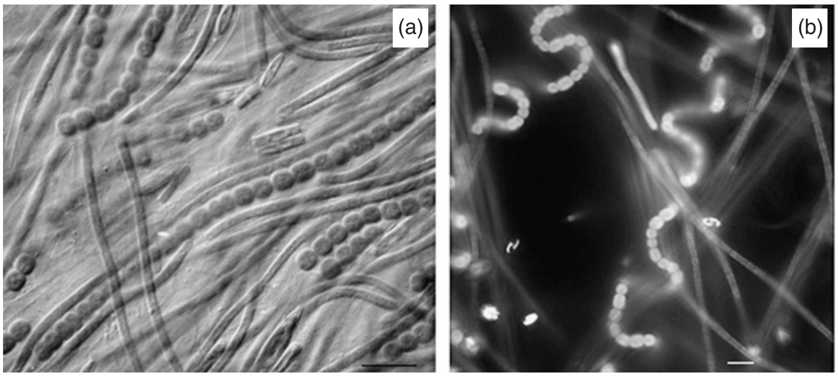

Fig 2: Bar equals 10m (courtesy of Roberta Congestri©)

of cyanobacterial and diatom external surfaces, was used to visualise these organisms better. This also promoted sediment desegregation, which facilitated microscopical observations of the morphological features and allowed taxonomical classification of bio-film components.

Thick, blue-green bio-films developed in cultures, with large portions adhering to vessel walls. Observations conducted with light (Figure 2a) and epifluorescence deconvolution microscopy (Delta Vision; Figure 2b) allowed a larger number of taxa to be identified as more diacritical features of cells became clearer in the cultured material. Confocal laser scanning microscopy (CLSM, Olympus FV1000) was also used in the preliminarily assessment of the three-dimensional structure of cultured bio-films.

In samples collected before *S. molesta* removal, photosynthetic microorganisms were distinguished among non-pigmented forms and abundant sediment particles. Filaments were attributed to cyanobacteria, photosynthetic prokaryotes (Anagnostidis and Komàrek, 1985; Komárek and Anagnostidis, 1989, 1999, 2005), while elongated symmetric unicells (provided by rigid, sculptured external investments) were identified as microalgae – more specifically, pennate diatoms (Cox, 1996).

Coccal cyanobacteria were identified as *Phormidium* sp. and *Leptolyngbya* sp., together with filamentous, non-heterocystous forms, whose cells were less than 3mm in width, approximately isodiametric or elongated. Pennate diatoms with bent frustules were tentatively identified as belonging to the genus *Eunotia*, while cells characterised by a bilateral symmetry were referred to as *Craticula* sp. and *Navicula* sp.

CLSM revealed thick and complex layered tangles of filaments and unicells in colonies. CLSM analyses also provided a better taxonomical characterisation of bio-film phototrophs, with over 11 taxa identified, including coccal and filamentous, non-heterocystous, cyanobacterial morphotypes, pennate diatoms and green algae.

Fig 3: A trap for invertebrates in a lateral cavity at the depth of 15m (courtesy of Roberto Palozzi©)

5.3. Zoology

During the period that *S. molesta* covered the water surface, sampling of invertebrate and vertebrate aquatic fauna was carried out applying scientific diving techniques: underwater direct observations; photo and video documentation; and placement of artificial traps in suitable spots at different depths. Micro-fauna traps were built by inserting a cut funnel spout in the opening of a small, plastic flask and keeping it balanced horizontally with weights. All the different parts were glued with liquid plastic to avoid the smell of traditional, synthetic glues. The traps were positioned along the sinkhole walls, in the middle of the water column every 3–5m with a rope and inside several lateral cavities (Figure 3); meat, fish and shrimp were used as bait. After the *S. molesta* was removed, divers also sampled invertebrate fauna and the newt population using nets from an inflatable dinghy.

In the Pozzo del Merro, four species of amphibian were recorded: *T. carnifex* (Italian crested newt); *L. vulgaris* (smooth newt); *Rana italica* Dubois (frog); and *Bufo bufo* L. (toad). Because the latter two amphibians were found only sporadically and the Italian crested newts largely outnumbered the smooth newts, *T. carnifex* was chosen as the focus and thus a study of its trophic niche was carried out.

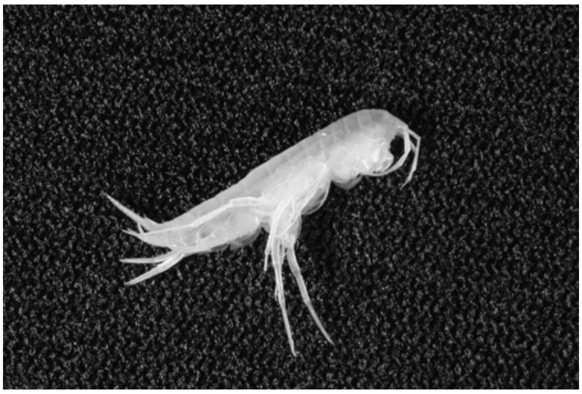

Fig 4: The endemic amphipod *Niphargus cornicolanus* (courtesy of Roberto Palozzi©)

After being captured, the newts were sexed and anaesthetised in tricaine methanesulphonate MS-222 (Novartis), which is one of the most commonly used anaesthetics for coldblooded animals and for amphibians in particular (Schumacher, 1996). Anesthetised newts were stomach flushed, which was repeated until no further content came out (Leclerc and Courtois, 1993). Entomological forceps were used to remove food items still present in the oral cavity after flushing.

Newts and frogs were released in the pond post-recovery, once normal activity was observed. No mortality occurred during or after stomach flushing. Taxonomic identification of stomach contents was performed using a stereomicroscope, and food items were identified to the lowest taxonomical level possible (often to family level). Underwater observations and photography confirmed the presence of both newt populations (with a large predominance of *T. carnifex*) dispelling the concerns about their survival after the fern invasion. Newts were observed diving no more than 4–5m deep. Divers also discovered a 23cm-long *Trachemys scripta* (Weid-Neuwied); an American aquatic turtle very popular in the aquarium market. It was possible that this released aquarium turtle could have been the *S. molesta* carrier.

Underwater traps captured only three Chironomidae (Diptera) in the first 12m, while four endemic amphipod, *Niphargus cornicolanus* (Iannilli & Vigna-Taglianti), were found between 12 and 20m deep. Only one of these was found in a trap inside a lateral cavity. During an exploratory dive, one specimen of *Niphargus* was manually collected at the depth of 74m (Figure 4).

Newts feed mainly on aquatic animals and, rarely, on terrestrial arthropods falling on the water surface. In particular, 95% of analysed stomach contents included preimaginal stages (i.e. larvae or pupae) of the moth *Cataclysta lemnata* L., whose larvae feed on *Lemna*. Preliminary results showed that the *Niphargus cornicolanus* is not included in the trophic niche of *T. carnifex*.

After the *S. molesta* removal, observations suggested that the ratio between smooth newts and crested newts was unbalanced. *T. carnifex* were largely predominant in the sinkhole, with the ratio of *L. vulgaris* to *T. carnifex* being 1:30.

The fact that very few invertebrate specimens had been collected, coupled with the evidence of still active newt populations under the *S. molesta*, resulted in the decision to remove the tropical fern promptly to avoid irreparable consequences for the aquatic fauna.

This preliminary study on the trophic niche of *T. carnifex* showed the importance of the autochthonous floating vegetation (which were substituted by the invasive *Salvinia*) because the main prey of the newts and the preimaginal stages of the lepidopteran *C. lemnata* are totally dependent on the duckweed (*Lemna* sp.), which was used as food or as a cocoon (at larval or pupa stage, respectively).

5.4. Archaeology

Archaeological surveys of the sediment in shallow water were led by manual inspection and an underwater metal detector. No traces of ancient human activities were found. It is very likely that the lack of human trace in the sinkhole was caused by the steepness of the walls. However, the surveys were not conclusive and did not preclude any kind of prehistoric or historic use of the sinkhole by human populations.

5.5. Diving physiology and pathology

It has been demonstrated in other studies that the main cause of injuries for SCUBA divers is inappropriate behaviour under stressful diving conditions, in particular emerging from elevated levels of anxiety with an increased prolactin level, which reflects a state of elevated physical and mental activity and vigilance (Anegg et al., 2002). It is known that the stress system is active when the body is at rest, responding to many distinct circadian, neurosensory and limbic signals (Mastorakos et al., 2005).

This study evaluated the hormonal response to stress during dives in a claustrophobic environment like the Merro sinkhole, which is a dark-water karstic cavity (Klimchouk, 2009). The stress induced by this very challenging dive was studied by comparing a famous free-diving champion (Stefano Makula, 53 years old and 28-time holder of the apnea world record) who dived in the well down to 48m, and three well trained SCUBA divers (aged 33, 58 and 51). All subjects were healthy men, and according to the Helsinki declaration, informed

Fig 5: Venous blood sample collected from the diver while still in the water (courtesy of Roberto Palozzi©)

consensus was obtained by all subjects involved in the study.

Venous blood samples were collected before training programme (basal condition) and during three different immersions performed in three different days, directly on the platform and often with the diver still in the water (Figure 5). A 10mL blood sample was collected by standard venipuncture immediately before and after each immersion. Of that, 7mL were placed in dry Vacutainer tubes (Becton Dickinson) to determine cortisol and prolactin concentration and 3mL in ethylenediaminetetraacetic acid (EDTA) Vacutainer tubes to determine adrenocorticotropic hormone (ACTH) values. Samples were centrifuged at 3000rpm for 15min and processed by routine clinical laboratory methods (Modular E, Roche). All blood sample collections were taken after at least a 12hr fasting period. Spirometry examination and oxygen saturation monitoring were performed on the steep slope inside the sinkhole just above water surface in a specific recess.

Preliminary analysis indicated that ACTH, cortisol and prolactin increased in all SCUBA and apnea divers tested after any immersions and most were over basal values (Figure 6). This seems to suggest an activation of hypothalamic-pituitary-adrenal (HPA) axis. Although the endocrine response to psychological stressors has been extensively studied, only a few studies have evaluated this response during stressful situations for SCUBA divers. This study recorded an increase in ACTH, cortisol and prolactin after immersions, and indicated a state of elevated physical and mental activation and vigilance even when the subjects were expert SCUBA and apnea divers. Thus an unusual and claustrophobic environment may cause activation in HPA axis (Mastorakos and Pavlatou, 2005) in all divers. The plasma prolactin increase has been described in recreational SCUBA diving (Rousseau et al., 2006) and, in this study, appeared in the apnea subject. Therefore, these variables seem to be useful as putative markers of environmental stress in human subjects.

5.6. Psychology

More than 50% of experienced divers have stated that they have suffered a panic attack at least once during their ''diving career'' (Polani, 1999) and panic may account for 20–30% of fatal accidents (with the possibility that it is the major cause of mortality in recreational underwater activities; Polani, 2004). Therefore, the claustrophobic and extreme underwater environment inside the Merro sinkhole provided a unique opportunity to study the diver's ability to manage the activation of the HPA axis and anxiety correctly.

Diver psychological profiles, before and after the dives, were obtained with the Profile of Mood States (POMS), a psychometric test from Educational Testing Service (EdiTS; McNair et al., 1971). The divers completed an initial written test just before jumping in the water and the second immediately after they resurfaced, though still in the water. POMS is questionnaire-based comprising 58 words or sentences having reference to six transitory dimensions of frames of mind: tension/anxiety; depression/disheartenment; aggressiveness/anger; vigor/activity; fatigue/slackness; confusion/abashment; and measures dimensions of the subject that are defined as transitory, i.e. closely connected to the event in a given period of time.

Psychological tests showed that the free-diving subject, though having expressed many times a sense of general fear of immersion into ''a frightening black hole'', carried out an optimal and safe performance, probably because of a highly professional preparation, physiological control, suitable techniques of mental training and pre-dive discussions with a psychologist.

Figure 7 represents the psychological situation before and after the free dive. Figure 7a (before the dive) shows that the diver was highly activated and, therefore, deeply psyched up (V of vigour); nevertheless, high levels of anxiety, even if under control, are noticeable (T of tension and D_1 of discouragement). After the dive, data change radically, as shown by the opposite trend of Figure 7b, which highlights the total relaxation of both attention and emotion at the end of the underwater performance, as a sort of emotional release. This indicates the total relaxation that a subject carrying out extreme physical exertion experiences when on return to ordinary life. In this case the free-diver trained himself with *ad hoc* techniques specifically tailored for him that allowed the emotional control of the anxiety

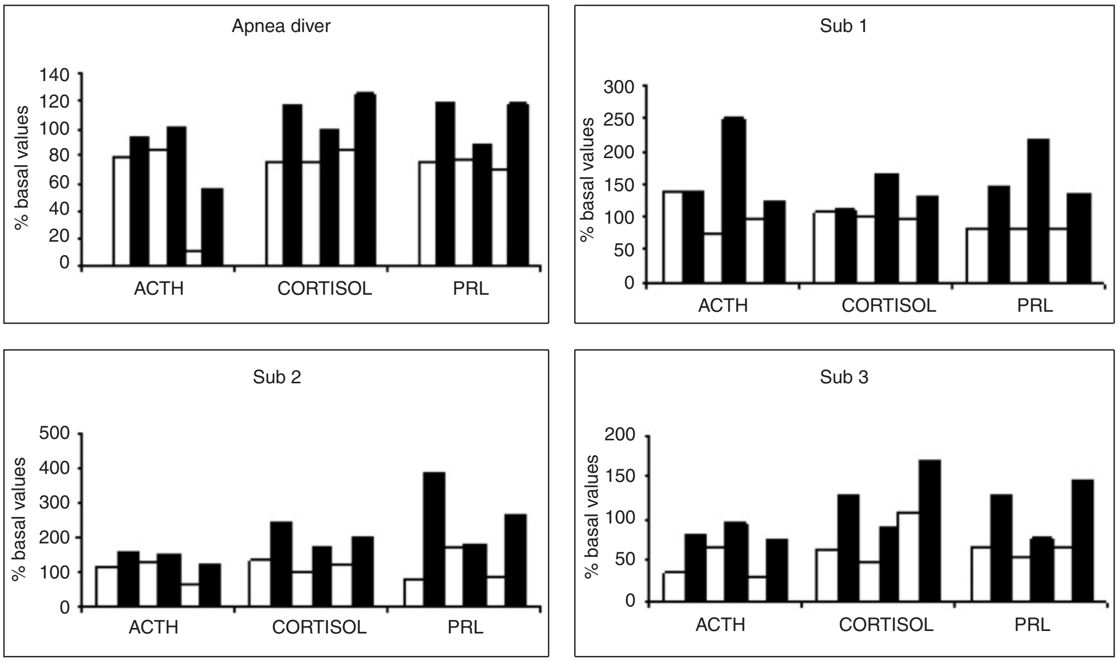

Fig 6: Hormonal parameters variation, expressed in percent of basal values, before (white bar) and after (black bar) three different dives which went progressively deeper

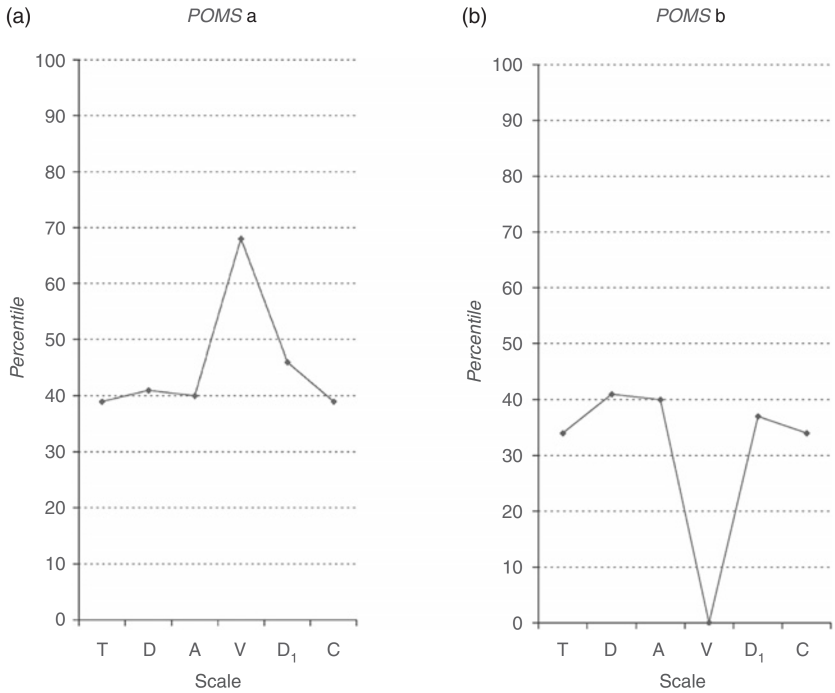

Fig 7: POMS graphs of expert apnea diver before (a) and after (b) dive

without compromising his concentration for the underwater performance.

6. Conclusions and recommendations

The Pozzo del Merro is a natural monument that needs to be studied more extensively both to guarantee its preservation and to take advantage of its unique place as a natural piezometer, an extraordinary link between the external environment and the ground-water resource. The MSP began to shed light on this ecosystem, but full knowledge of it is still far from reach. The role of scientific diving will be crucial in this difficult challenge.

The first analyses revealed a 'hidden' microflora colonising the limestone walls of the Merro sinkhole, unveiling a source of biodiversity in this system within an extreme environment in terms of its peculiar hydrology and light regime. Thus, a multiphasic approach was used to better assess taxon richness of microphytobenthic communities and their relationships with phytoplankton and floating plants. It also contributed towards evaluating the sinkhole's potential for hosting rare species, typical of extreme and confined habitats and endemism.

The preliminary observations on the samples collected after *S. molesta* removal suggested that the modified ecological conditions are leading to a new range of microphytobenthic communities. In addition, the discovery of the introduced turtle gave one potential explanation for the mystery of the fern arrival. Restricting any further invasions will help to retain the unique ecosystem within the sinkhole.

Before this study, no information was available for the newt syntopic population, and their interactions in the study site were completely unknown. Additionally, the occurrence of an endemic species of crustacean, *Niphargus cornicolanus*, which was thought to be a potential newt prey, could instigate a larger and more thorough study on the trophic niche occupied by these amphibians and on the implications for their conservation and management.

With regard the medical research, this preliminary study might represent the basis for future work on the physiology of immersions in obstructed environments. In particular, it might be of great interest to determine new biochemical pathways of stress caused by the extreme environmental conditions, such as the claustrophobic environment of a dark water sinkhole. There is a growing population of cave divers and there may be a requirement to better understand the range in individual limitations (Polani, 1999, 2004). The study's psychological tests underlined that the main difference between a well trained diver and an unpractised one is in the ability to weigh up the risk state with extreme tranquillity. A well trained diver clearly knows risk is a personal limit function that can be managed by one's own technical abilities.

Acknowledgements

This study was authorised and financially supported by the Provincia di Roma – Assessorato all'Ambiente. We also want to thank Mondo Sommerso; Corpo Forestale dello Stato – Scuola Allievi Agenti di Cittaducale; Coltri Sub; Dive System; Nimar; and Associazione Nazionale Istruttori Subacquei for their tecnical support. We are very grateful to Ivan Gnan, Pierluigi Boccanera, Ernesto Bianchetti, Massimiliano Falleri, Marco Arnaud, Biagio Pino e Christine Scott for their help. A special thanks is also given to Jim Bowden.

References

Abbate E. (1890). *Guida della Provincia di Roma.* Roma: CAI Sezione di Roma.

Anagnostidis K and Komàrek J. (1985). Modern approach to classification system of cyanophytes. 1 - Introduction. Arch Hydrobiol. *Algological Studies* **38/39**: 291–302.

Anegg U, Dietmaier G, Maier A, Tomaselli F, Gabor S, Kallus KW and Smolle-Jüttner FM. (2002). Stress-induced hormonal and mood responses in scuba divers: a field study. *Life Sciences* **70**: 2721–2734.

Balcombe FG, Cordinglej JN, Palmer RJ and Stevenson RA. (1990). *The Cave Diving Group Manual: Cave Diving.* Somerset, UK: Mendip Publishing, 268pp.

Brubakk AO and Gutvik C. (2006). Optimal decompression from 90 msw. In: Lang MA and Smith NE. (eds.). Proceedings of Advanced Scientific Diving Workshop, 23–24 February, Smithsonian Institution, Washington, DC.

Caramanna G. (2001). Scientific utilization of scuba diving and ROV techniques in an inland flooded sinkhole in the Latium region (Central Italy) for the hydro geological and geochemical study of the karst water resource. Volume dei riassunti ''Geoitalia 2001- 3° Forum italiano di Scienze della Terra'' (Chieti, 5–8 Settembre 2001), 302–303.

Caramanna G. (2002). Exploring one of the world's deepest sinkholes: the Pozzo del Merro (Italy). *Underwater Speleology* **29**: 4–8.

Casale C, Maniscalco A and Toro B. (1963). Su alcune forme carsiche nei Monti Cornicolani. In: *Atti del V Congresso degli Speleologi dell'Italia centrale.* Terracina, 41–47.

Casati L. (2007). *Manuale di Speleologia Subacquea.* Florence: Olimpia, 204pp.

Chiocchini M and Mancinelli A. (1978). Ricerche geologiche sul Mesozoico del Gran Sasso d'Italia (Abruzzo). III. Correlazioni tra le facies di margine della piattaforma carbonatica e facies pelagiche del Giurassico e Creatceo inferiore Studi Geologici Camerti n. 4.

Chrousos GP. (1995). The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *New England Journal of Medicine* **332**: 1351–1362.

Cox EJ. (1996). *Identification of Freshwater Diatoms from Live Material.* Oxford: Chapman and Hall, 168pp.

Davis JC and Elliott D. (1982). Treatment of decompression disorders. In: Bennett PB and Elliott D. (eds.). *The Physiology and Medicine of Diving.* Third edition. London: Bailliere-Tindall, 475–476.

Egstrom GH. (2006). Historic perspective: scientific deep diving and the management of the risk. In: Lang MA and Smith NE. (eds.). Proceedings of Advanced Scientific Diving Workshop, 23–24 February, Smithsonian Institution, Washington, DC.

European Community Commission. (2008). Verso una strategia comunitaria per le specie invasive. Comunicazione della Commissione al Consiglio, al Parlamento Europeo, al Comitato Economico e Sociale Europeo e al Comitato delle Regioni. SEC (2008)2887 and SEC (2008)2886.

Farr M. (2003). *Diving in the Darkness: Beneath Rock, Under Ice, into Wrecks.* Cardiff: Publishing Wild Place, 128pp.

Francis TR, Dutka AJ and Hallenbeck JM. (1989). Pathophysiology of decompression Sickness. In: Bove AA and Davis JC. (eds.). *Diving Medicine.* Second edition. Chapter 15. Philadelphia: WB Saunders.

Gary M, Sharp J, Caramanna G and Havens R. (2003). Volcanically influenced speleogenesis: forming el sistema Zacaton, Mexico and Pozzo del Merro, Italy, the deepest phreatic sinkholes in the world. In: Proceedings of the Geological Society of America Annual Meeting, 2–5 November, Seattle, Washington. 19-4.

Giardini M and Caramanna G an Calamita U. (2001). L'imponente sinkhole del Pozzo del Merro (Monti Cornicolani, Roma): stato delle conoscenze. *Natura e Montagna* **42**: 12–27.

Giardini M. (2003). Note sulla biologia, l'ecologia e le modalità di controllo di *Salvinia molesta* D.S. Mitchell (Salviniaceae), specie infestante nuova per il Lazio. *Rivista di Idrobiologia* **42**: 263–282.

Giardini M. (2004). *Salvinia molesta* D.S. Mitchell (Salviniaceae): seconda segnalazione per l'Italia (Lazio) e considerazioni sul controllo di questa specie infestante. *Webbia* **59**: 457–467.

Iannilli V and Vigna Taglianti A. (2005). New data on genus *Niphargus* (Amphipoda, Niphargidae) in Italy, with the description of a new species of the *Orcinus* group. *Crustaceana* **77**: 1253–1261.

Kindwall EP and Whelan H. (eds.). (1999). *Hyperbaric Medicine Practice*, 2nd edition. Flagstaff: Best Pub Co., 955pp.

Klimchouk A. (2009). Morphogenesis of hypogenic caves. *Geomorphology* **106**: 100–117.

Komárek J and Anagnostidis K. (1989). Modern approach to the classification system of cyanophytes. 4 – Nostocales. *Archiv fuer Hydrobiologie Supplement* **82**: 247–345.

Komárek J and Anagnostidis K. (1999). Cyanoprokaryota, 1. Teil. Chroococcales. In: Ettl H, Gärtner G, Heynig H and Mollenhauer D. (eds). *Süßwasserflora von Mitteleuropa Band 19/2.* Jena: Gustav Fischer, 1–523.

Komárek J and Anagnostidis K. (2005). Cyanoprokaryota, 2. Teil. Oscillatoriales. In: Büdel B, Gärtner G, Krienitz L and Schagerl M. (eds.). *Süßwasserflora von Mitteleuropa.* Munchen: Elsevier GmbH, 1–720.

Lang MA. (2009). Parameters of extreme environment diving. In: Lang and Brubakk. (eds.). Proceedings of the Future of Diving: 100 Years of Haldane and Beyond. Washington, DC: Smithsonian Institution Scholarly Press, 153–160.

Leclerc J and Courtois D. (1993). A simple stomach flushing method for ranid frogs. *Herpetological Review* **24**: 142–143.

Mastorakos G and Pavlatou M. (2005). Exercise as a stress model and the interplay between the hypothalamus pituitary-adrenal and the hypothalamus-pituitary-thyroid axes. *Hormone and Metabolic Research* **37**: 577–584.

Mastorakos G, Pavlatou M, Diamanti-Kandarakis E and Chrousos GP. (2005). Exercise and the stress system. *Hormones* **4**: 73–89.

Mattei M, Montone P and Salvini F. (1986). Analisi strutturale dei rilievi del margine appenninico intorno a Tivoli (Roma). *Memorie della Scoietà Geologica Italiana* **35**: 579–589.

Maxia C. (1954). La geologia dei Monti Cornicolani (Lazio). Istituto di geologia e paleontologia dell'Università di Roma, Pubbl. n.11, Roma, 111pp.

McNair D, Lorr M and Droppleman LF. (1971). *Manual for the Profile of Mood States.* San Diego, CA: Educational and Industrial Testing Services, 27pp.

Moon RE. (1996). Which initial recompression treatment? In: Treatment of Decompression Accidents in Recreational Diving. 2nd European Consensus Conference, Marseille, 9–11 May 1996, 116–125.

Mount T and Gilliam B. (1993). *Mixed Gas Diving: The Ultimate Challenge for Technical Diving.* San Diego, CA: Watersport Publishing, Inc, 392pp.

Polani D. (1999). *Psicologia dell'immersione.* Florence: Olimpia, 96pp.

Polani D. (2004). Verso una nuova didattica: risultati di una ricerca. 1° Congresso Internazionale dell'Associazione Italiana di psicologia Subacquea, Siracusa, 19–22 May.

Rousseau AS, Richer C, Richard MJ, Favier A and Margaritis I. (2006). Plasma glutathione peroxidase activity as a potential indicator of hypoxic stress in breath-hold diving. *Aviation, Space, and Environmental Medicine* **77**: 551–555.

Schumacher J. (1996). Reptiles and amphibians. In: Lumb WV and Jones EW. (eds.). *Veterinary Anaesthesia*, 2nd edition, Philadelphia: Williams and Wilkins, 670–685.

Schwerzmann M and Seiler C. (2001). Recreational scuba diving, patent foramen ovale and their associated risks. *Swiss Medical Weekly* **131**: 365–374.

Segre AG. (1947). Bacino imbrifero e zona carsica del Pozzo del Merro nei Monti Cornicolani. Notiziario del Circolo Speleologico Romano, n.5.

Segre AG. (1948). I fenomeni carsici e la speleologia del Lazio. Pubblicazioni dell'Istituto di Geografia dell'Università di Roma, Serie A, N. 7, 61–67.

Southerland DG. (2006). Medical fitness at 300 FSW. In: Lang MA and Smith NE. (eds.). Proceedings of Advanced Scientific Diving Workshop, 23–24 February, Smithsonian Institution, Washington, DC, 92–100.

Tuccimei G. (1886). Considerazioni sopra il Karst-phänomen dei Monti Sabini. *La rassegna Italiana, Anno VI* **II**: 85–99.