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Survival, metabolic rates and locomotory activities of a groundwater-obligate copepod species under long-term exposures to tetrachloroethylene

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1. INTRODUCTION

Tetrachloroethylene (TCE; C_2Cl_4) is a contaminant frequently found in groundwater bodies of industrialized areas worldwide. In most Europe, the quality threshold values for TCE is 1.1 µg/L (concentration of no environmental concern) for groundwater bodies.

Obligate groundwater-dweller organisms (stygobionts) have developed adaptive traits to survive in total darkness, in photosynthesis-lacking habitats where organic matter is scarce and supplied from the surface. These animals are blind, small-sized, colorless and with low metabolic rates. They quickly respond to habitat disturbances and are particularly sensitive to chemical stressors.

In this study, we investigated the effect of a 5-day exposure to 1.1 µg/L TCE on the survival, oxygen consumption and locomotory activities of a stygobiotic copepod (Crustacea, Harpacticoida) species (*Moraria* sp.; Fig. 1), endemic of Antro del Corchia Cave in the Apuan Alps (Tuscany, Italy).

2. SAMPLING SITE

Monte Corchia karst system is located in the south-western part of the Apuan Alps (NW Tuscany, Italy; Fig. 2) and is developed inside the carbonate core of a syncline, almost completely enclosed by a low-permeable basement. Corchia Cave is about 60 km long and 1185 m deep and it is presently one the largest and deepest caves in Italy. The presently known cave consists of a 3D network of passages distributed from 1640 to 450 m a.s.l., having the shape of regular tubes or canyons and with a mainly horizontal pattern. Several high-gradient passages form the present active drainage system from the surface to the main collector. The system now has 20 entrances accessible to humans (Fig. 3).

3. MATERIALS AND METHODS

Specimens of *Moraria* sp. were collected from the vadose zone of the cave, located between 900 and 800 m a.s.l., which consists of several phreatic passages that are part of the touristic path since 2001. The water from ceiling drip (Fig. 4) was directed through a funnel into a plastic container. A 2 cm by 3 cm area on each of two sides of the square container was cut out and covered with a net (mesh size 60 µm) to retain animals (Fig. 5).
We measured the individual-based oxygen consumption of this species as a proxy of possible metabolic reactions to long-term (5 days) exposures to TCE at 8.0°C, i.e. about the mean annual temperature of groundwater in the cave. To this end, we used a sealed glass microplate equipped with planar oxygen sensor spots with optical isolation glued onto the bottom of 80-µL wells (Loligo Systems, Denmark) integrated with a 24-channel fluorescence-based respirometry system (SDR Sensor Dish Reader, PreSens, Germany). The system allows simultaneous measurement of 20 replicates and 4 blanks. Survival assessments were performed by counting the number of alive individuals at the end of the exposure. The locomotory activity was measured as the number of moving animals in 5 mL glass vials each containing 20 individuals after the exposure, where the movement was manually recorded over 4 minutes followed by an interval of 6 minutes for 1 hour (Fig. 6).

4. RESULTS AND DISCUSSION

Survival rates were 100% both in the control and in the treatment with TCE. No significant difference in the locomotory activity was assessed. Prosome length, width and the dry mass of the individuals used in the control were not significantly different from those in the treatment (p-value = 0.3074). The standard respiration rates (SRR), measured as µg O_2 per individual per hour, were significantly lower (p-values = 0.0023) in the treatment (0.0073 ± 0.0054) than in the control (0.0211 ± 0.0153), as shown in the Fig. 7a,b.

5. CONCLUSIONS

This is the first study about the respiratory impairment of a stygobiotic copepod species under long-term exposure to a sub-lethal concentration of a widely diffuse organic pollutant. Our findings show that TCE does not affect either the survival or the locomotory activity of *Moraria* sp. On the contrary, it induces a reduction of the respiratory activity, whose significance should be further investigated. Is it a physiological or a pathological response to a hazardous compound's concentration that is considered of no environmental concern for EU groundwater bodies?

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6. ACKNOWLEDGMENTS

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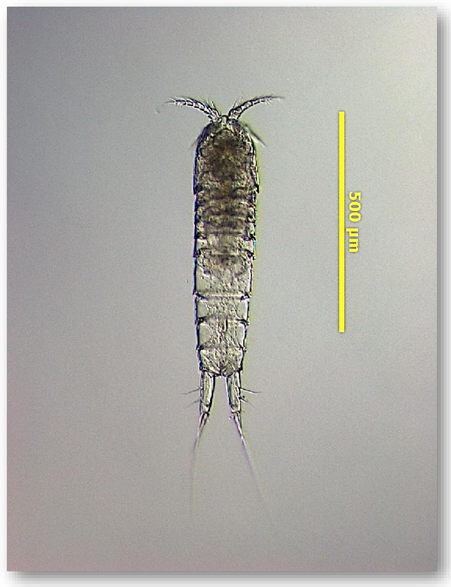


Fig. 1. Female of *Moraria* sp.

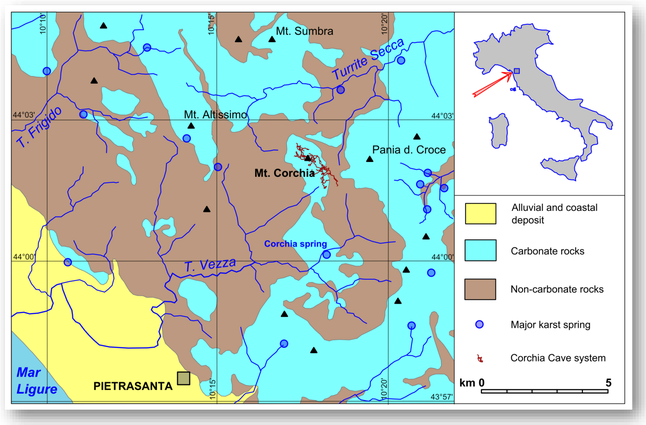


Fig. 2. Location of Monte Corchia karst system.

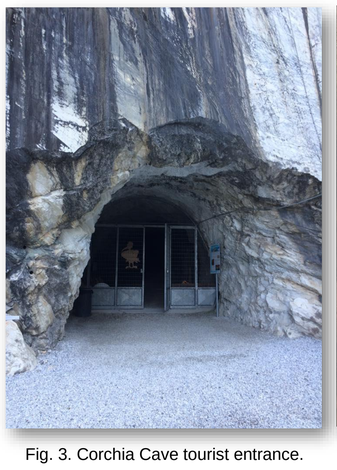


Fig. 3. Corchia Cave tourist entrance.

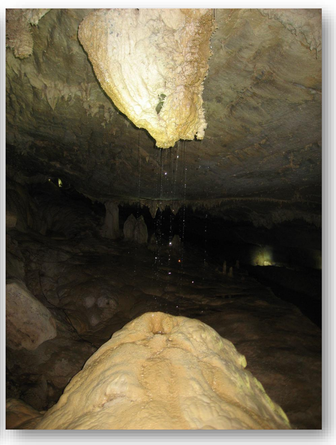
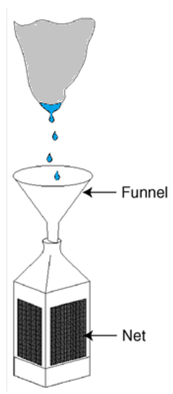


Fig. 4. Corchia Cave ceiling drip.



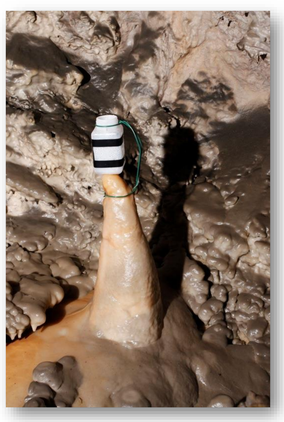


Fig. 5. Sampling device for collecting the ceiling drip and retain animals.

Fig. 6. Locomotory activity measurements.

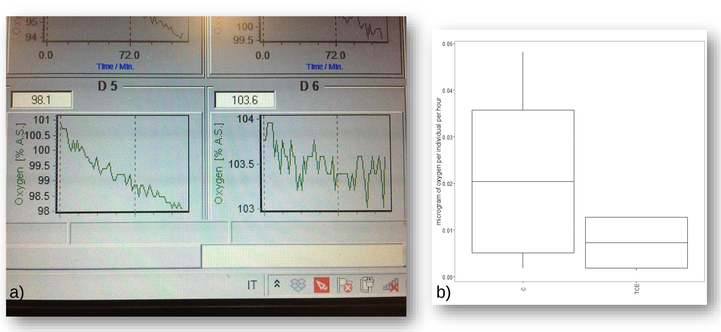


Fig. 7. a) Oxygen consumption of an individual of *Moraria* sp. in the control (D5) and in the TCE treatment (D6). b) Boxplot showing the significant difference between the mean oxygen consumptions of *Moraria* sp. in the control and in the TCE treatment.