# Ciliated protozoa of a geothermal sulphur spring

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# Abstract

The species richness and the dynamics of the ciliate communities were studied at three stations along a streamlet formed by water discharged from a geothermal sulphur spring. Altogether, 73 species were identified. Ciliate coenoses were composed primarily of freshwater forms, but they were numerically dominated by euryhaline species. The great fluctuation in both number of species and individuals observed along the streamlet were due to vigorous chemical changes in the physico-chemical characteristics of the water. These changes were caused mainly by the sulphide and salt gradient observed along the water course. The maximum value of sulphide was observed in August with 391  $\mu$ M 1<sup>-1</sup>, and the highest conductivity value was observed in April with 18 mS cm<sup>-1</sup>. The most characteristic and abundant species were small-sized bacterivorous scuticociliates which have a rapid ecological adaptability and whose tolerance limits to environmental changes are wide. Bacterivorous scuticociliates such as *Cyclidium citrullus*, *C. glaucoma*, *Cinetochilum margaritaceum*, and *Uronema nigricans* were numerically dominant when the thermal water contained the highest concentration of sulphide. The diversity index values changed sharply in time both inside and among sampling stations, indicating unstable communities which depended on highly fluctuating conditions.

# Introduction

Protozoa are characteristically phagotrophic, especially on bacteria, unicellular algae and other protists, and their phagotrophy underpins their ecological importance in microbial food webs. In the open water of lakes and oceans (Berninger et al., 1991) and in anoxic sediments (Fenchel & Finlay, 1990), they are quantitatively the most important consumers of other microorganisms. Heterotrophic protista have been studied in lakes, rivers, and oceans, and numerous reports have noted the importance of these organisms as links between heterotrophic bacteria and zooplankton grazers (e.g. Pace, 1982; Azam et al., 1983; Rublee & Partusch-Talley, 1995). In fact, these organisms are intermediate in remineralization and in recycling of essential nutrients (Sherr & Sherr, 1984). Moreover, protista play an important part in systems of prediction and assessment of water quality (Bick, 1968; Sladecek, 1973). These organisms have several advantages for this purpose because they combine biological and functional mechanisms in a single cell, have a higher growth rate, can be maintained easily in laboratory conditions, have a cosmopolitan distribution, are very sensitive to toxic substances, and represent a remarkable proportion of the biomass in aquatic environments (Cairns, 1982; Dale, 1991).

Among protista, ciliates are organisms which are abundant in fresh waters and in some cases constitute the dominant group. Numerous papers have been published on their biology, ecology and incidence in the saprobity of natural waters. The interest of these studies lies not only in taxonomic knowledge of these organisms but in their distribution and response to the variations of their natural habitat conditions. However, very little attention has been paid to the exotic habitats such as inland saline waters (Stiller, 1963; Wilbert, 1995), acidic lakes (Bienert et al., 1991; Järvinen, 1993), and sulphide-containing springs of geothermal origin (Stiller, 1946; Pax, 1948).

The specific objectives of this study were to characterize the ciliate community that colonized a little stream receiving waters from a geothermal spring containing sulphide, and to determine the response of these organisms to sulphide gradients during a one-year sampling period.

#### Materials and methods

#### Study site

Bobbio's thermal resort is located at 2.5 km from Bobbio (44° 37' N, 9° 24' E), a small town in the province of Piacenza (Northern Italy), on the right side of the Trebbia river. Geothermal waters are drawn up from a depth of 25 m and piped to the thermal resort. Thermal waters not utilized are piped into the Trebbia pebble river-bed where they run in a pebbly streamlet 200-m long before to flow into the Trebbia river waters. Bobbio's thermal waters are classified as 'sodio-sulphureous waters' and are athalassic saline, with Na<sup>+</sup>, S<sup>--</sup>, and Cl<sup>-</sup> as the predominant ions, and with a salinity range of 0.6–9.9‰. In winter the thermal waters result to be diluted with rainfall waters that penetrate in the thermal aquifer in consequence of the increased piezometric level of water table. Owing to the small flow (on an average of 16 liters  $s^{-1}$ ), water level in the streamlet was of few centimeters only (5– 7 cm).

Samples were collected monthly from February 1995 to January 1996 from three stations along the streamlet. Station 1 was located at the beginning of the streamlet 1 meter afterwards water inlet; station 2 was located about in the middle of the stream run, and station 3 was located few meters before the streamlet flows into the Trebbia river.

#### Physico-chemical environment

Water temperature and dissolved oxygen were measured *in situ* using a bulb thermometer and the Winkler method, respectively. Water samples were taken in each station and filtered (Whatman GF/C filters) to be analysed for pH, conductivity, salinity, and sulphide, according the Standard Methods (APHA, 1985). Dissolved sulphide was determined following the method suggested by Cline (1967).

### Sampling and enumeration of protista

Samples were collected at the water-sediment interface with a 4 cm diameter pump aspirator, specifically designed for sampling streams characterized by hard bed and low water level, and already used in previous studies (Madoni & Rossi, 1975). At each station three samples were collected along the line of the stream cross-section and pooled. Samples were examined in the laboratory and the abundance ratios were determined within five hours of collection. In most cases, ciliated protozoa were classified in vivo. When necessary, samples were prepared for microscopical examination with silver carbonate staining (Fernandez-Galiano, 1976). Ciliophora were identified according to Kahl (1930-35) and Foissner et al. (1991–1995). Other protists than ciliates, and metazoa were not identified but some characteristic forms were assigned to generic level following Lee et al. (1985) and Streble & Krauter (1981). Estimates of population density were based on enumerations from subsamples extracted with an automatic micropipette. The most appropriate drop size and number of replicate counts were selected according to the subsampling technique described by Madoni (1984). Mostly, sub-samples of 100  $\mu$ l in volume were taken and ten replicates of this volume were counted. The density of each taxon was expressed as number of individuals per 1 cm<sup>2</sup> of bottom area.

### Results

#### Physical and chemical factors

Seasonal changes of physical and chemical factors along the streamlet are summarized in Figure 1. These factors showed a similar trend in the three sampling stations but it was observed a gradient in their values among stations. In fact, conductivity and sulphide decreased from station 1 to station 3. Dissolved oxygen and pH values, on the contrary, were higher at station 3 than at station 1. Values of pH ranged from 7.3 (st. 1) to 8.0 (st. 2 and 3). At the three stations, water was characterized by quite steady thermal values around 15 °C during the studied period. Nevertheless, a maxima of 22 °C was observed in April, and a minima of 8 °C was registered in January. At the three stations, maximum values of dissolved oxygen were almost all found during winter with the minima found in summer. These values varied between 4.0 and 10.1 mg  $1^{-1}$ . The conductivity is raised by the geological nature of the underground. Maximum values (18 mS cm-l) were found in summer (station 1), while minimum values were found during winter when thermal waters were