Temporal variability of the cyanobacterium *Oscillatoria sp.* in extreme environment -a sulfide-rich hot spring outflow

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Introduction

Los Baños de la Hedionda (Andalucía, southern Spain) is a sulfide-rich hot spring outflow (Fig. 1). While sulfide is appreciated in thermal bath, sulphide is toxic to most oxygenic photosynthetic organisms because it irreversibly blocks photosystem II. In spite of being a site of public use, and provide an interesting case to conditions for investigate on extremophile organisms, little is known about the temporal variability of the sulfide concentration and photosynthetic organisms inhabiting this extreme ecosystem. Here we present the first annual cycle of phytoplankton composition related to sulfide concentration.

Material and methods

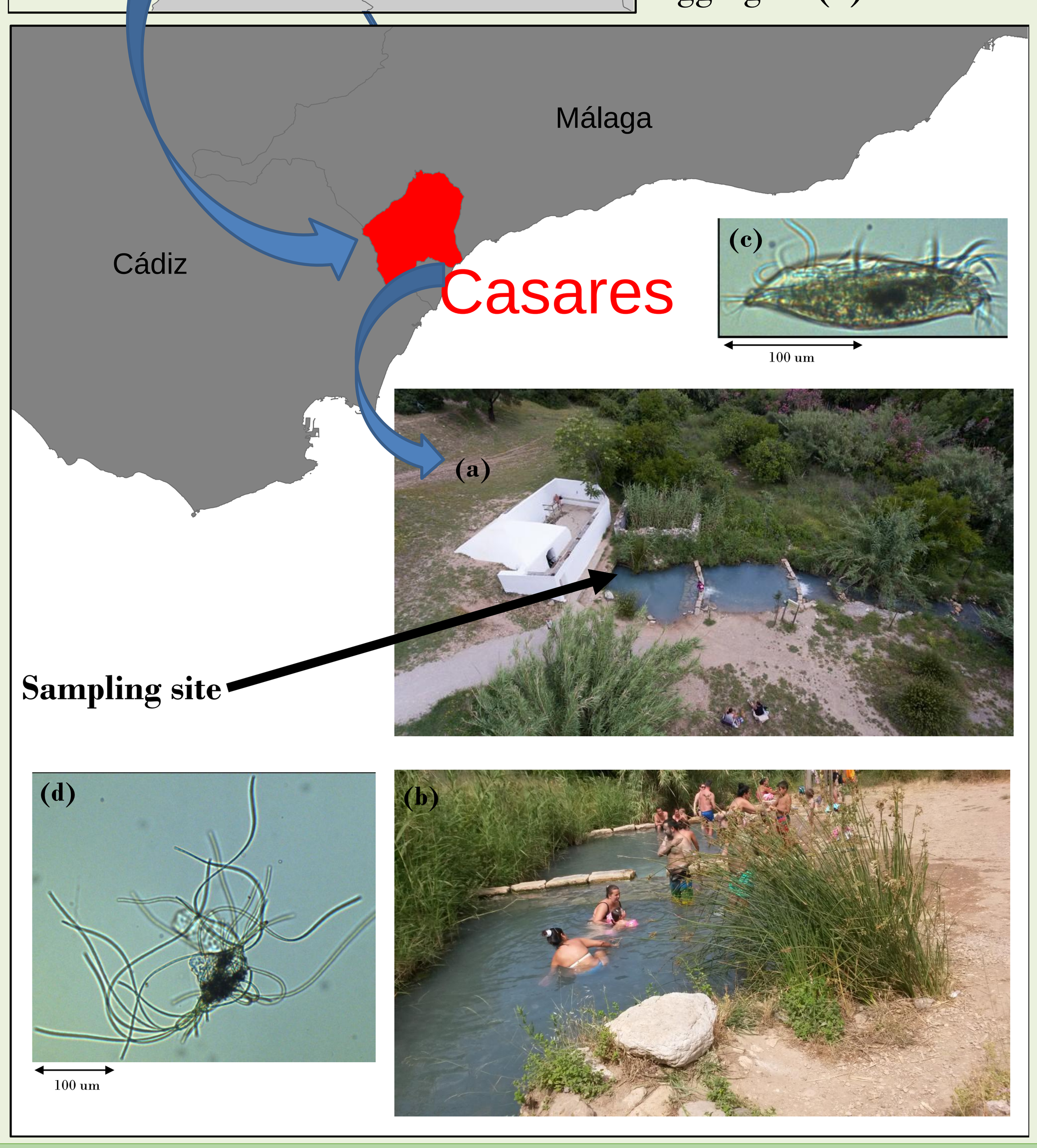

Fig. 1 Location of the sulfide-rich hot spring outflow "The Hedionda". Sampling site (a) and public use of the sulfide–rich outflow during summer (b). Ciliate (c) and *Oscillatoria sp.* ciliate aggregate (d)

Monthly and weekly field sampling was carried out between 11/03/2016 and 30/05/2017.

At each sampling day, pH and sulfide concentration were measured in situ with a (Hanna HI 9125 and DR900 Multiparameter Portable Colorimeter respectively). Then, 5L samples were taken in the morning (9:00-11:00h), placed in polyethylene bottles and maintained in cold and dark during the 1h transport to the laboratory at the University of Málaga.

At the laboratory, total chlorophyll a concentration and phytoplankton composition were measured with a submersible fluorospectrometer (BBE-Moldaenke FluoroProbe). The fluorospectrometer discriminated between the main phytoplanktonic groups (i.e. diatoms and dinoflagellates, cyanobacteria, green algae and cryptophytes) based on the relative fluorescence intensity of chlorophyll a (Chl a) at 680 nm, following sequential light excitation by 5 Light-Emitting Diodes (LEDs) emitting at 450, 525, 570, 590 and 610 nm [1-2]. For abundance and size estimation of *Oscillatoria sp.*, 2L of sample were concentrated on a 45m mesh and recovered in 20ml. Then each sample was passed through the FlowCAM equipped with a 100 µm flow cell and a 100-fold magnification (10x objective).

The analysis was performed in autoimage mode, where individual pictures of each particle in the vision field are taken. Afterwards phytoplankton abundance, size and biovolume were estimated by manual reprocessing of the original data fields [3]. Meteorological data were downloaded from the meteorological sampling station of Estepona [4].

Discussion

Sulfide, chlorophyll a concentration and *Oscillatoria sp.* abundance and biovolumen show a clear annual cycle, with a hot, dry and sulphide-rich period (June-November), and a colder, sulfide-poor period (December-May). Low sulfide concentration is related to dilution by recharging of the aquifer in winter and spring. It is worth to note that highest Chl a concentration as well as highest *Oscillatoria sp.* abundance were found during the period where toxic sulfide concentration was highest. Low Chl a concentration during low sulfide concentration period could be due to higher cell loss of phytoplankton by water runoff as could be compensated by growth rate. However, algal loss by runoff would affect all planktonic groups, but the other taxa remain in similar concentration during the whole year (<1-2 mg Chl a /L). **Which advantages explain the success of *Oscillatoria sp.*?**

Physiology: Growth rate as a function of sulfide concentration is shown in poster 656, by Martín-Clemente et al. (2017) and could explain increasing *Oscillatoria sp.* abundance in summer.

Morphology: Taking into account that the other phytoplanktonic groups are not filamentous and small sized (<45 µm), filamentous algae and aggregates (*Oscillatoria sp.*) might get trapped on other water plants and branches giving *Oscillatoria sp.* an advantage in a fluvial system.

Trophic interactions: Apart of phytoplankton we also found some ciliates (Fig. 1c), specially in summer, which might control smaller sized phytoplankton. Images from fresh samples passing through the FlowCAM show Ciliate-Oscillatoria aggregates (Fig. 1d). Thus ciliates might also predate on *Oscillatoria sp.*, however big-sized *Oscillatoria sp.* aggregates could prevent top down control until elevate ciliate abundance, which might occur under elevate sulfide concentration (>200 µM), as runoff should be slower and trophic interaction tighten as residence time increases.

Conclusions

Sulfide concentration of the Hedionda is around 40µM in winter and above 100µM in Summer. *Oscillatoria sp.* is the predominant alga throughout the year, but highest concentrations are linked to sulfide concentration between 100-200µM. This could be due to (i) advantages in growth rate at elevate sulfide concentration (poster 646), (ii) avoiding top down control by greater sized aggregate building and (iii) diminishing algal loss in a fluvial system by getting trapped on plants and branches.

References

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Results

Temporal evolution of mean air temperature an radiation (Fig. 2) shows an annual cycle where radiation proceed temperature. Temperatures above 20ºC were registered between June and November.

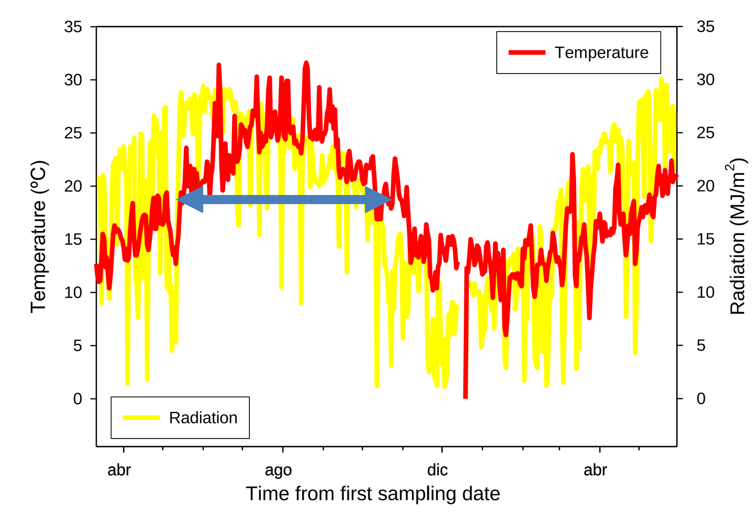

Fig. 2 Temporal evolution of mean air temperature and radiation during the sampling period. (↔ Time period of temperature >20ºC)

Precipitation (Fig. 3) was absent in summer (June-September) and some small precipitation were observed in October-November, before considerable precipitation occurred in December.

Sulfide concentration (Fig. 3) higher than 100 µM were observed during the warm (>20ºC) and dry season, which dropped down to 40 µM after the registration of the first precipitations. Low sulphide concentration maintained from December to May, and increased again in the last sampling date (30/05/2017).

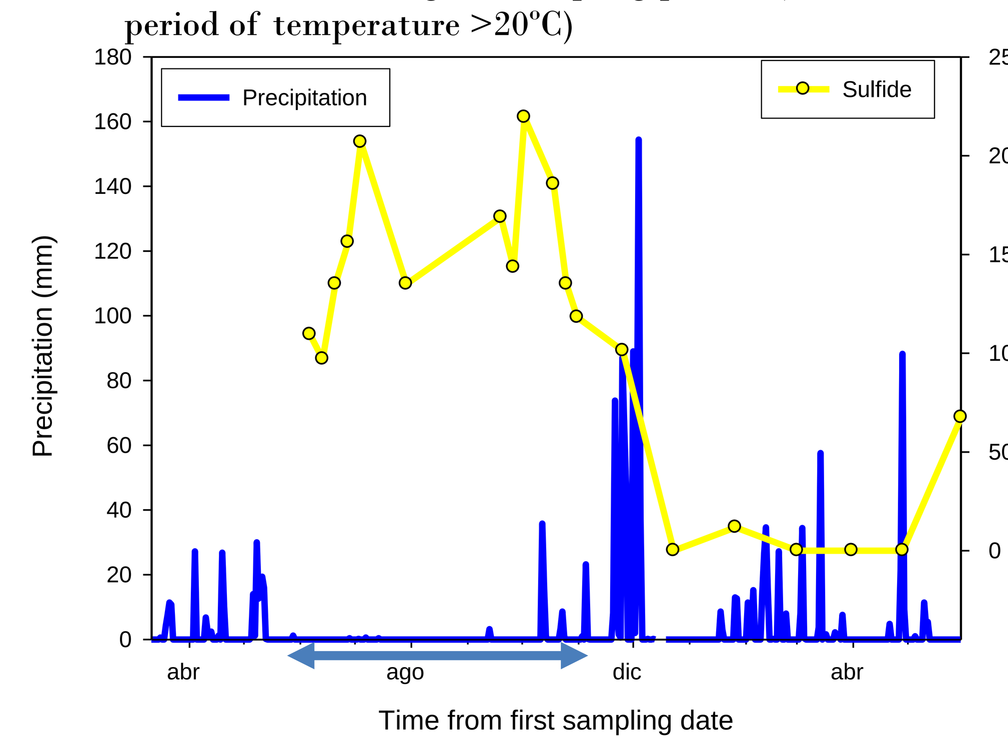

Fig. 3 Temporal evolution of precipitation and sulfide concentration in the samples of hediond.
(↔ dry, sulfide-rich season)

Highest chlorophyll a concentration (11 µg Chl a /l) was observed at (27/06/2016, Fig. 4). In general Chl a concentration was significant higher (Median=1.8µg/l) during the dry season when sulfide concentration was above 100 µM, than when it was bellow 100µM (Median=0.5µg/l) (P<0.004 Mann-Whitney Rank Sum Test). Phtoplankton community was dominated by bluegreen algae (in our case *Oscillatoria sp.*) throughout the year, and specially during the dry, sulphide-rich season (80% of chlorophyll corresponded to bluegreen algae).

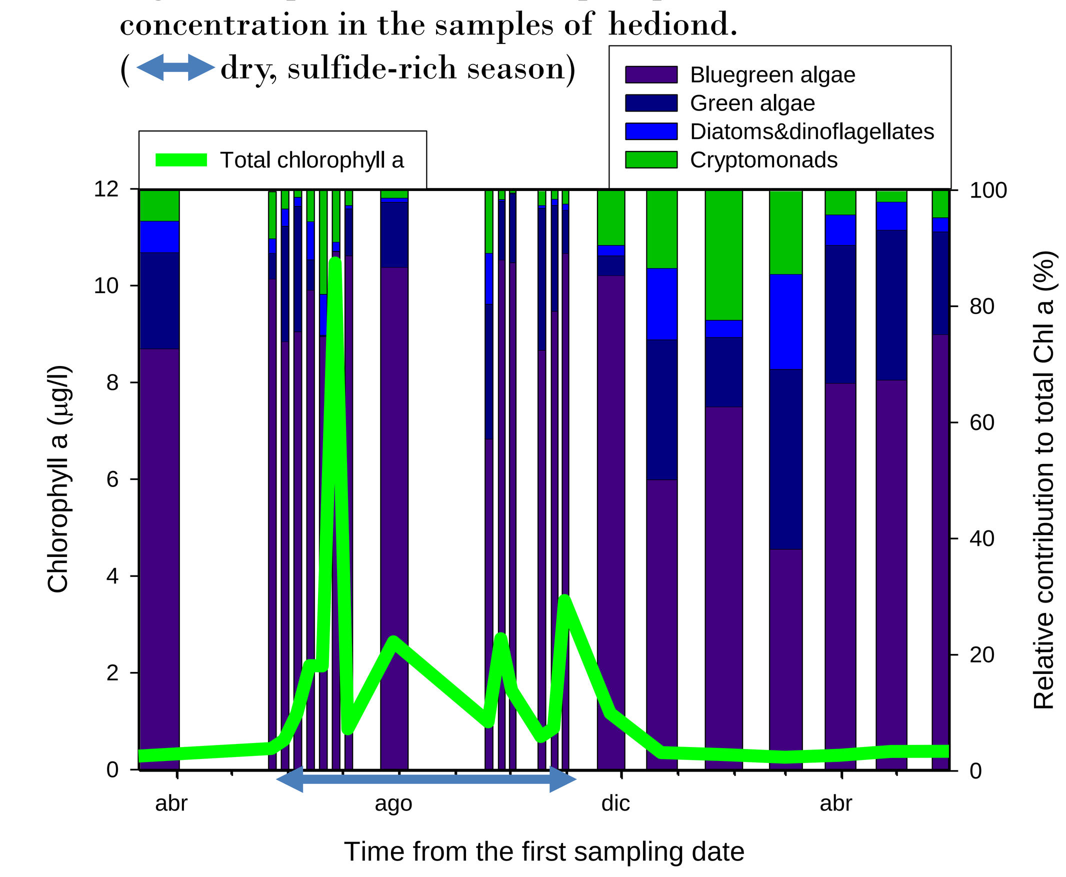

Fig. 4 Temporal evolution of total Chl a concentration and relative contribution of different groups discriminated by fluorescence fingerprint. (↔ dry and sulfide rich season).

Abundance and biovolume of *Oscillatoria sp.* (<100µm) was highest at sulfide concentration between 100 and 200 µM (Fig. 5). Considering the whole planktonic community (including aggregates of *Oscillatoria sp.* >100µm), highest concentration of *Oscillatoria sp.* was observed at sulfide concentration between 100 and 200 µM.

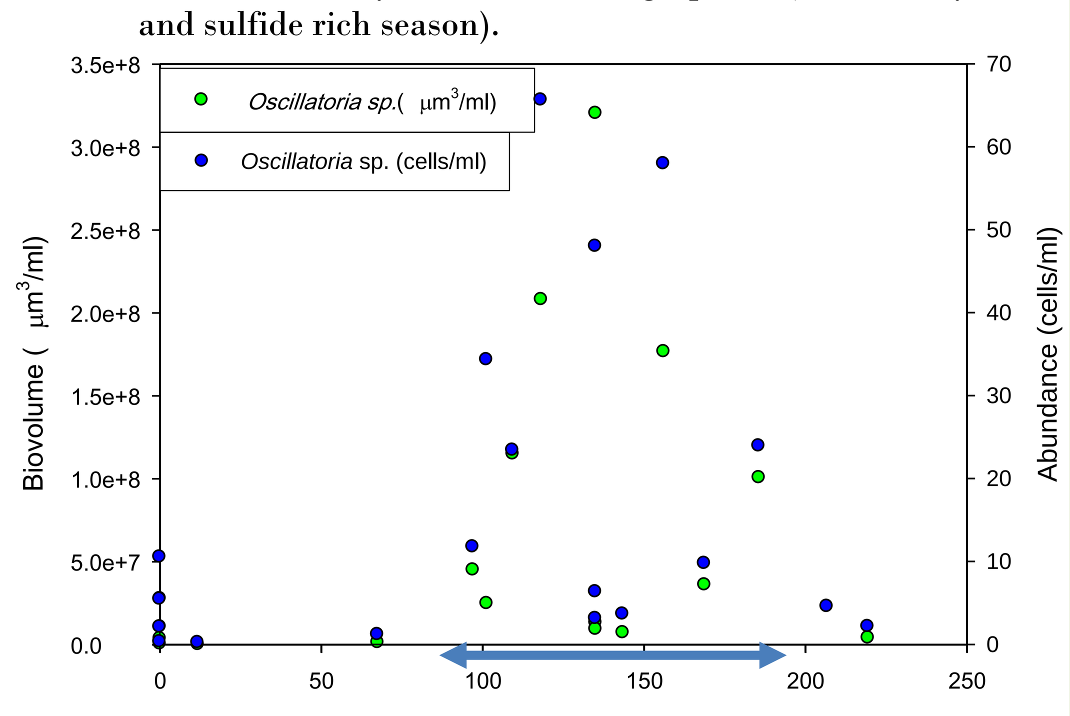

Fig. 5 Biovolume and abundance of *Oscillatoria sp.* versus sulfide concentration.

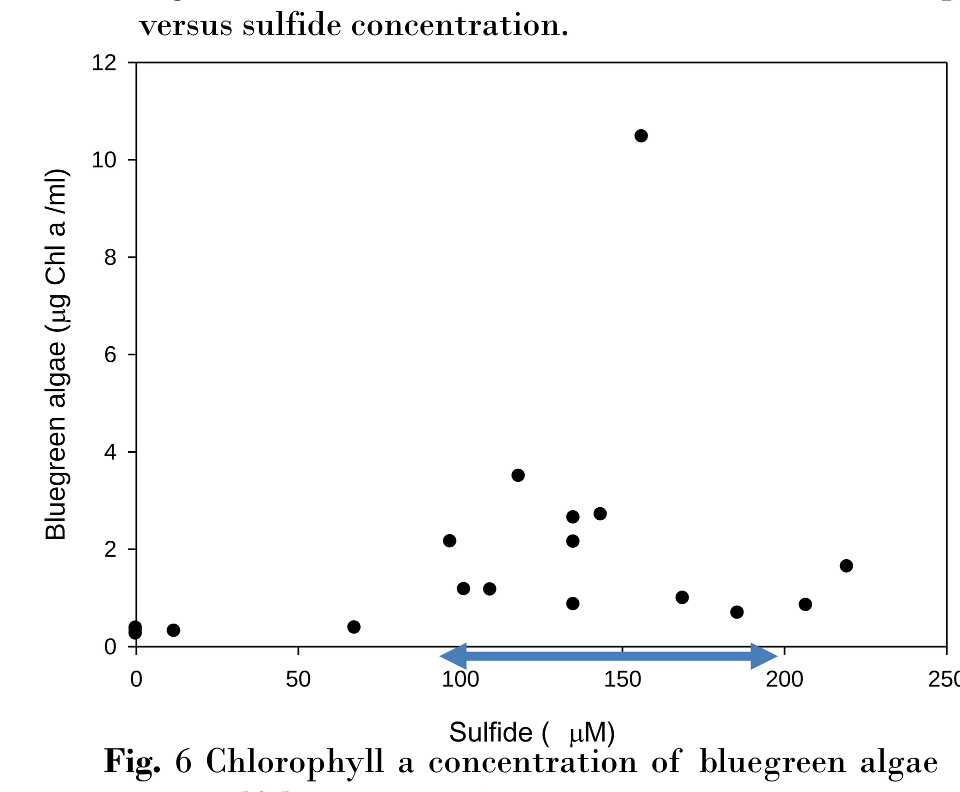

Fig. 6 Chlorophyll a concentration of bluegreen algae versus sulfide concentration.

Acknowledgments

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