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Research papers

The anoxic stress conditions explored at the nanoscale by atomic force microscopy in highly eutrophic and sulfidic marine lake

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A B S T R A C T

Marine Rogoznica lake (RL), an eutrophic and euxinic environment situated at the eastern Adriatic coast was used as a natural laboratory to test the application of atomic force microscopy (AFM) in combination with the electrochemical and high temperature catalytic oxidation (HTCO) measurements for characterization of water column organic matter (OM) and reduced sulfur species (RSS) in relation to seasonal changes of environmental conditions. Water column of RL was explored at the nanoscale by the AFM during the anoxic holomictic event (S1, October 2011) and stratified winter (S2, January 2012) and spring (S3, May 2012) conditions in the oxic and anoxic water samples. Obtained results from the AFM uphold the electrochemical and HTCO measurements, indicating significant difference in the present type of OM during the holomictic, anoxic stress conditions in comparison to the samples collected during the stratification period. Differences in the OM type were discussed in line with the physical and biological processes that occurred in RL during sampling: mixing processes characterized by fast turnover of water layers and biological activity characterized by low (January 2012) and high (May 2012) primary production of diatoms and zooplankton grazing activities.

1. Introduction

Oxygen deficiency i.e. hypoxia or anoxia occurs throughout the world in the coastal waters and areas of the coastal ocean where oxygen is low or absent in bottom waters. In many modern coastal systems, anthropogenic changes are superimposed on natural variation and increased input of nutrients from anthropogenic sources affect the natural systems, enhancing the algal blooms, and increase in OM flux (Eldridge and Roelke, 2011; Paerl, 2006). Therefore, so-called dead zones are expanding worldwide nowadays. The sinking of OM and subsequent decay leads to high demand in oxygen what can cause periodic or permanent water column hypoxia (0.2 mg/l < $[O_2]$ < 2mg/l), anoxia ($[O_2]$ < 0.2 mg/l) or even euxinia ($[O_2]$ < 0.2 mg/l, free $[HS^-]$) (Eldridge and Roelke, 2011; Neretin, 2006). Anoxia normally occurs in enclosed basins (including fjord-type estuaries, seawater lakes, and anchialine caves) where physical barriers and density stratification limits the advection of O_2 to the deep waters and remineralization processes enhance deposition of the OM and nutrients in the water column and sediments (Eldridge and Roelke, 2011; Neretin, 2006).

RL, also known as the Dragon Eye, is a unique example of small and shallow (circular shape with an area of 10.276 m^2, maximum length of 143 m, and a maximum depth of 15 m in the middle of the lake), euxinic and eutrophic marine environment, situated on the Gradina Peninsula at the eastern coast of the Adriatic Sea (43°32′N, 15°58′E middle Dalmatia, Fig. 1a) (Ciglenečki et al., 2015 and references therein). Due to the high phytoplankton activity upper part of the water column is well oxygenated while remineralization processes enhance deposition of organic matter (DOC up to 6 mg/l), and nutrients (NH_4^+, up to 315 μM; PO_4^{3-}, up to 53 μM; and SiO_4^{4-}, up to 680 μM) in hypoxic/anoxic part of the water column where milimolar concentration of sulfide is confirmed (this study and Kršinić et al., 2000; Ciglenečki et al., 2005, 2015; Bura-Nakić et al., 2009; Žic et al., 2013; Pjevac et al., 2015 and references therein). Existence of a strong thermohaline and redox conditions i.e. chemocline can be found at the oxia/anoxia boundary. Around chemocline, a layer with dense population of sulfide oxidizing bacteria (SB) mainly genus *Chromatium* (purple SB, PSB) and *Chlorobi* (green SB, GSB) is situated. Population density of GSB is much higher but due to the size of individual bacterial PSB cell, this layer has a purple color (Ciglenčki et al., 1998; Pjevac et al., 2015). There is no direct connection between RL and surrounding sea, however tides are visible on the rocks, what indicates water intrusion through porous carbonate (Žic et al., 2013). The lake is under strong influence of

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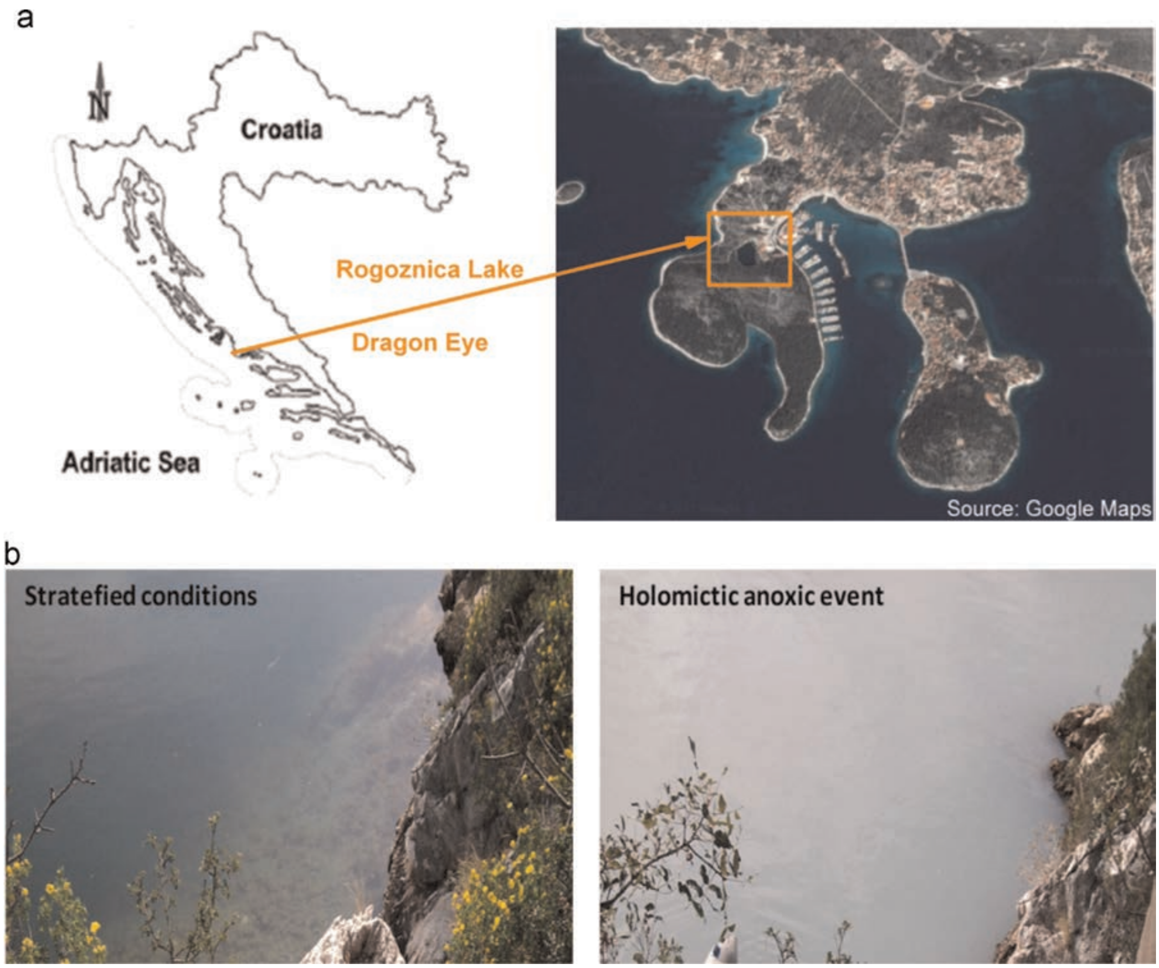

Fig. 1. (a) Map indicating position of RL and (b) photo of the RL surface water taken during holomictic and stratified conditions. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

atmospheric i.e. meteorological conditions (Ciglenečki et al., 2015) which influence stratification and water column stability, as well position of chemocline. The only input of fresh water to RL is through precipitation.

The mixing between water layers depends on the meteorological conditions which highly influences its intensity and usually can be detected in autumn as a result of termohaline dissolution and stratification breakdown inducing holomictic conditions. If the typical stratification breakdown is accompanied by the fast turnover of the water layers, triggered by extreme weather events (Ciglenečki et al., 2015), fast transport of highly sulfidic bottom water to the surface may cause consumption of all dissolved oxygen and occurrence of anoxic conditions in the whole lake (Bura-Nakić et al., 2012; Ciglenečki et al., 2005). Under these circumstances the presence of different reduced sulfur species (RSS – sulfide, polysulfide and colloidal sulfur formed by oxidation) and relatively high nutrient concentration can be found throughout the water column of RL (Bura-Nakić et al., 2009, 2012; Žic et al., 2013; Ciglenečki et al., 2015 and references therein). Rapid precipitation of colloidal sulfur influence change in the lake's surface color, which in contrast to stratified conditions when the water transparency is high, is non-transparent milky-yellowish (Fig. 1b). If the mixing process is relatively slow, driven only by the termohaline dissolution and vertical diffusion, oxygenated surface waters can diffuse to the bottom anoxic layer, or anoxic bottom waters rich in sulfide and nutrients can diffuse to the surface. In this case the mixing process can last up to 7 days, and may result in oxygenation of the entire water column (Ciglenečki et al., 2015). Upward, transport of ammonium which is facilitating biologically mediated nitrification is considered to be primarily source of high nitrate concentrations (up to 30 μM) that can be usually detected in the entire water column during winter and spring months. The eutrophication of the lake is strongly influenced by nutrient recycling under anaerobic conditions (Žic et al., 2013; Ciglenečki et al., 2015 and references therein).

Seasonal variation of basic physico-chemical parameters (temperature, salinity, and dissolved oxygen) measured in RL water column from middle of the lake, during 2011 and 2012 are presented in Fig. 2, where stratification breakdown and establishment of holomixis and new stratification cycle are clearly visible.

During the last 20 years of our research activities in RL (1994 – up to day), the anoxic and sulfidic holomictic events were followed twice, in September 1997 and October 2011 (Bura-Nakić et al., 2009, 2012; Ciglenečki et al., 2015). In both occasions high sulfide and ammonium concentrations that were detected through the entire water column, lead to mass mortality of all phytoplankton and benthic organisms (Kršinić et al., 2000; Barić et al., 2003; Ciglenečki et al., 2005). Mass mortality contributed to increased nutrient concentration in RL, even in the surface layer that remained high for several months after holomictic anoxic event due to remineralization processes, decomposition of large amount of particulate organic carbon (POC) and lack of intensive primary production (Kršinić et al., 2000; Barić et al., 2003; Ciglenečki et al., 2005).

Influenced by the work of the Svetličić group who introduced