Seasonal Variation of Anoxic Conditions in the Rogoznica Lake*

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Seasonal variations of temperature, salinity and concentration of dissolved oxygen, as well as vertical distribution of dissolved organic matter, surface active substances, phytoplankton and reduced sulfur compounds in the small, intensely eutrophicated, 15 m deep sea-lake, Rogoznica Lake, were investigated during 1994, 1995 and 1996. Anoxic conditions in the Lake and high concentrations of sulfur compounds (up to 10^{-4} M, mainly in the form of sulfide) were detected below the 9 m depth in the periods of stable stratification in 1994 and 1995. At the boundary between oxic and anoxic conditions (chemocline), the presence of purple sulfur bacteria (genus *Chromatium*) and a maximum concentration of thiosulfate (8×10^{-6} M) were detected. The stratification and mixing of lake water were greatly influenced by rainfall, as shown from decreased salinity in deeper waters. High phytoplankton activity over the entire water column and an extremely high production of oxygen (oxygen saturation up to 300%) probably prevented the expected development of anoxia during the spring of 1996.

INTRODUCTION

Anoxic, sulfide-bearing bottom waters characterize many lakes, marine basins, and fjords with thermo-haline stratification.[1,2,3,4,5] Low oxygen concentrations occur in such waters when oxygen utilization rates exceed the

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oxygen replenishment rates across the formed pycnocline, which prevents vertical mixing. In the marine environment, oxygen consumption is closely connected with oxidation of organic matter and the dissolved oxygen, used for this oxidation, is derived from photosynthetic processes and from the atmosphere.[6] When the supply of free oxygen nears exhaustion, oxidation of organic matter is continued *via* sulfate reduction and sulfide production, which are diagnostic indicators of anoxic conditions.[7] Anoxic waters are characterized by a strong chemocline (here defined as the interface between oxygen and sulfide) which is accordingly a zone of high chemical and micro-
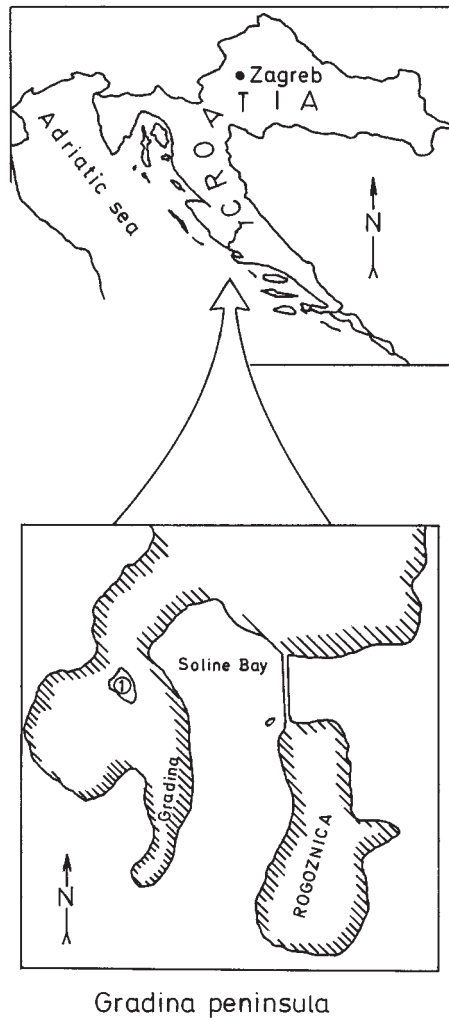

Figure 1. Geographical position of the Rogoznica Lake.

bial activity.[2,8,9] Dense populations of photoautotrophic bacteria are common in many stratified waters where light penetrates into the sulfide layers.[1,10,11] In nearly all cases, these are purple or green sulfur bacteria, which photo-oxidize sulfide to elemental sulfur and sulfate.

The Rogoznica Lake is a small, intensely eutrophicated sea-lake, situated on the eastern coast of the Adriatic Sea (Figure 1). The Lake is surrounded with sheer cliffs (4 – 23 m high). It has an area of about 5300 m^2 and a maximum depth of 15 m. The Rogoznica Lake is a periodically anoxic basin.[12] Vertical mixing occurs during winter when cold, oxygen-rich water from the surface sinks downwards. The Lake has no visible connection with the surrounding sea but lake tides are detectable on the cliffs, which indicates that underground water connection exists. Despite permanent water exchange between the Rogoznica Lake and the surrounding sea through the porous karst,[13] anoxic conditions prevail in deeper layers of the Lake, probably due to remineralization of organic matter produced in the period of intensive primary production. During thermo-haline stratification in 1994 and 1995, surface water was well oxygenated, while the layer below 9 m depth was anoxic.[14,15] Anoxic deep water was rich in sulfur (up to 900 μM), especially in the form of sulfide or elemental sulfur.[14] The distribution of iodine species was found to be strongly influenced by the occurrence of anoxic conditions in the Lake.[15] Phytoplankton was composed of a relatively small number of species, which were mostly distributed above the 10 m depth. The heterotrophic dinoflagellate *Hermesinum adriaticum* was mostly distributed arround the oxic-anoxic interface of the Rogoznica Lake.[16]

As distinguished from previously published paper on the Rogoznica Lake, in which preliminary results of sulfur speciation were presented only in two sampling periods (April 1994 and 1995),[14] this study describes seasonal variations of oxic-anoxic conditions, as well as the vertical distribution of dissolved organic matter, surface active substances (SAS), phytoplankton cell-density and sulfur compounds in the lake water column during 1994, 1995 and 1996. Also, special attention was paid to the distribution of different sulfur species along the vertical profile (sulfide, elemental sulfur, thiosulfate and polysulfide).

EXPERIMENTAL

Sampling and Analyses

Water samples from the Rogoznica Lake were collected with 5 l Niskin sampling bottles in April and July 1994, April 1995, and at approximately monthly intervals between October 1995 and October 1996, along the vertical profile of the Lake. Water samples for oxygen and sulfide measurements were always taken first from the Niskin bottles immediately after the sampler was back on board. To preserve anoxic conditions, the Niskin sampler was attached to a N$_2$ cylinder to maintain N$_2$

over water during sample collection from the Niskin sampler through a tube into glass bottles. The bottles had been previously flushed with N_2, and were filled with samples to overflowing. Temperature and salinity were measured *in situ* with a Hg-thermometer and refractometer (Atago, Japan).

Samples for sulfur analyses were measured unfiltered and fresh, within 24 h.

Samples for surface active substances (SAS) and dissolved organic carbon (DOC) measurements were stored in dark glass bottles. Nonfiltered lake water samples were analyzed for SAS within 24 h. DOC analysis was performed on samples which were filtered immediately after sampling (Whatman GF/F filters – pore size 0.7 μm), poisoned with $HgCl_2$, and stored in a cold dark place for measurements.

Samples for phytoplankton analysis were preserved in 2% (final concentration) neutralized formaldehyde solution.

Chemical Analyses

Electroanalytical method of cathodic stripping a.c. (alternating current)[14,17] and linear sweep voltammetry[18] were used for the determination of sulfur species (sulfide, polysulfide, elemental sulfur, thiosulfate) in the lake water, according to electrode reactions presented in Table I.

TABLE I

Reactions of sulfur species at the mercury electrode[18]

HS^-	$+$ Hg	$\Leftrightarrow$	$HgS + H^+ + 2e^-$	$E_{1/2} = -0.68$ V
S^0	$+$ Hg	$\Leftrightarrow$	$HgS_{ads} + 2e^-$ $\Leftrightarrow$ $Hg^0 + S^2$	$E_{1/2} = -0.68$ V
S_x^{2-}	$+$ Hg	$\Leftrightarrow$	$HgS + (x-1)S + 2e^-$	$E_{1/2} = -0.68$ V
$2\,S_2O_3^{2-}$	$+$ Hg	$\Leftrightarrow$	$Hg(S_2O_3)_2^{2-} + 2e^-$	$E_{1/2} = -0.12$ V

Electrochemical measurements were performed with a Methrom Polarographic analyzer E-506 connected with HMDE (Methrom) as the working electrode (for cathodic stripping a.c. voltammetry), and with μ–Autolab Electrochemical Instruments (Eco Chemie, the Netherlands) connected with 663 VA Stand Methrom electrode for linear sweep voltammetry. The reference electrode was an Ag/AgCl (1 M NaCl) electrode, and a platinum wire served as the auxiliary electrode.

Cathodic stripping a.c. voltammetry was used for (in phase) measurements at the frequency of 75 Hz, at a.c. amplitude 10 mV, and potential scan rate 10 mV s^{-1}. LSV measurements were performed at 100 mV s^{-1} scan rate.

Potential scans in the negative direction were applied after accumulation of sulfur on the electrode surface by stirring the solution at two different starting potentials: $E = -0.2$ V and $E = 0$ V *vs*. Ag/AgCl, for 0 and 120 s, respectively. Measurements were performed in a 50 cm^3 glass cell and, prior to each measurement, deaeration with purified nitrogen for 3 min was undertaken. The measurements were made at room temperature (293 K).

Sulfide was analyzed by changing experimental conditions (accumulation time) depending on the concentration. Low sulfide concentrations ($< 10^{-6}$ M) were measu-

red with accumulation by stirring at the starting potential, and high sulfide concentrations ($>10^{-6}$ M) were measured either without accumulation at the starting potential, or using dilution of the sample before measurement (maximum dilution was 1 : 500).

Analytical determination of elemental sulfur, sulfide and polysulfide was based on acidification of the sample with conc. HCl (pH = 2–3) and on elimination of sulfide with purging N_2.[14,17] It is known that thiosulfate ions also decompose to elemental sulfur and sulfite in acidic conditions. However, this is not a rapid process and during 24 h of thiosulfate incubation in acidic conditions only 1% of added thiosulfate gets decomposed.[19]

Electrochemical determination of thiosulfate is based on the reduction of $Hg(S_2O_3)_2^{2-}$ formed during the potential scan in the negative direction from 0 V to -1.0 V *vs.* Ag/AgCl.[19] Appearance of voltammetric peaks of sulfide and thiosulfate at different potentials enables their determination in the mixtures.

Oxygen concentration was determined by the standard manual Winklers method.[21]

The content of surface-active organic matter was determined by a.c. voltammetry (out of phase mode), and expressed as the equivalent amount of Triton-X-100, as described previously.[21] DOC measurements were performed using a Shimadzu TOC-500 Analyzer provided with a high-temperature oxidation technique.

Phytoplankton Counts

Cell counts were obtained by the inverted microscope method.[22] Subsamples of 25 and 50 ml were analyzed microscopically after sedimentation times of 24 h and 48 h, respectively. Microphytoplankton cells (MICRO, cells longer than 20 μm), including those of microheterotrophic species *Hermesinum adriaticum*, were counted under magnifications of 400x (1–2 transects) and 100x (transects along the rest of the counting-chamber base plate).

RESULTS AND DISCUSSION

During 1994 and in April 1995, anoxic conditions were detected in the water column of the Rogoznica Lake.[14] In this period the boundary between oxic and anoxic sulfur compounds rich water (up to 10^{-4} M, mainly in the form of sulfide) was at a 9 m depth. Investigations of lake water during 1996 showed that vertical thermo-haline gradients, as well as depth-positioning of oxic-anoxic inerface varied with the season. The salinity, temperature, concentrations of dissolved oxygen and total reduced sulfur in the Rogoznica Lake at the depth of 12 m also varied seasonally during 1994, 1995 and 1996 (Figure 2). At the 12 m depth, temperature ranged from 14 to 26 °C, and the salinity from 34 to 38. Oxygen concentrations varied between 0 and 0.40 mM, and were closely related to the concentrations of reduced sulfur species (10^{-2} to 10^{-8} M). During the period of very low oxygen concentration (below 0.05 mM) in 1994 and in April 1995, the concentration of total reduced sulfur was very high (concentration ranged from 10^{-4} to
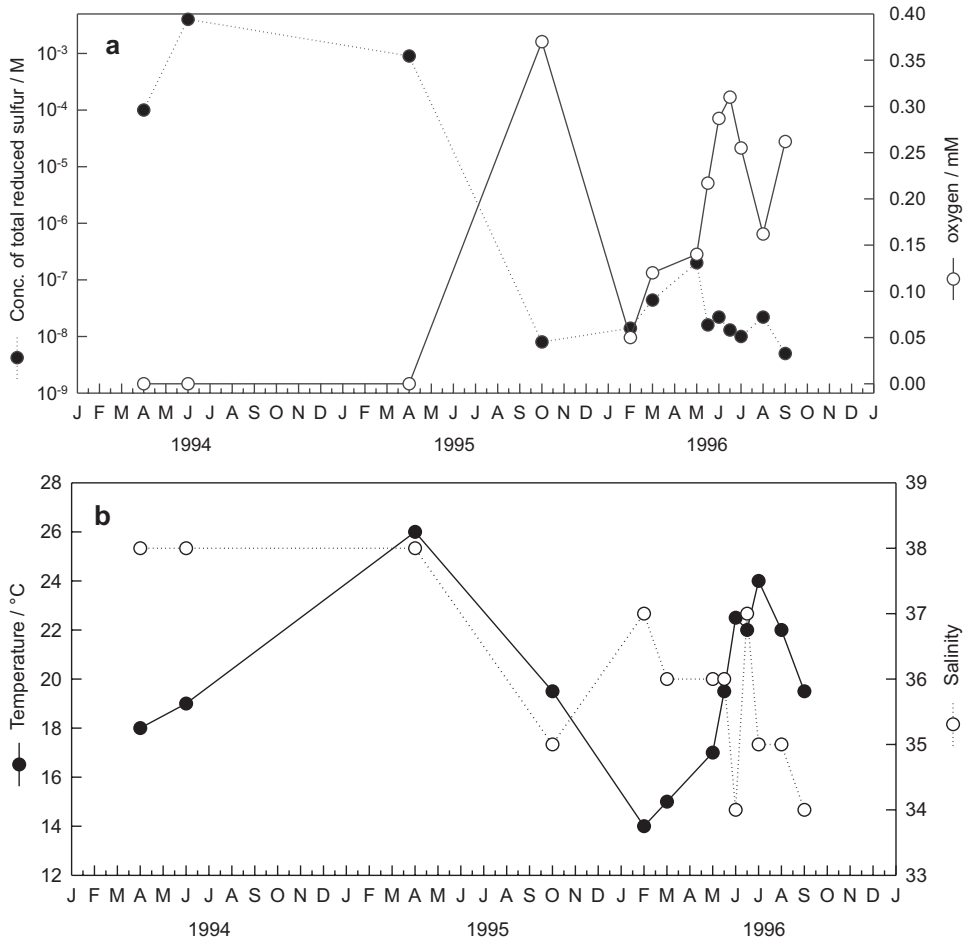
Figure 2. Seasonal variations of concentrations of oxygen and total reduced sulfur (a), and temperature and salinity (b) at the 12 m depth of the Rogoznica Lake.

10^{-2} M). At the same time, the salinity at the 12 m depth was stable and without water exchange with the surrounding coastal seawater and oxygen rich surface water. The salinity of the surface layer in the Rogoznica Lake changed seasonally depending on meteorological conditions and rainfall. Unusually large amounts of rainfall were observed in the area of Rogoznica in the autumn and winter of 1995, and during 1996 (especially in August). It can be presumed that the abundant rainfall as well as the cold winter 1995/1996 caused the prolonged mixing of lake water.

The influence of fresh water inflow is visible in the salinity decrease at the depth of 12 m, (Figure 2b). The mixing of lake water and the decline in salinity were accompanied with an increase in dissolved oxygen concentra-

tion and a decrease in reduced sulfur species. The concentration of total reduced sulfur at the 12 m depth had the same trend as that of salinity, indicating the strong influence of rainfall on the vertical salinity gradient and mixing of the water in the Rogoznica Lake.

To explain the exchange of oxic and anoxic conditions in the Rogoznica Lake, several different and characteristic biochemical situations in the Lake have been selected, as illustrated in Figures 3–5 and 7.

The vertical profile of sulfur compounds in October 1995 is shown in Figure 3B. Figures 3A, C and D present the concentrations of dissolved oxygen, DOC and SAS, and the gradients of temperature and salinity along the vertical profile of the Rogoznica Lake during the sampling period. According to the salinity, temperature and oxygen profiles (Figure 3A), the water column of the Lake was completely mixed and oxygenated with 0.37 mM oxygen at the depth of 12 m. The salinity values along the vertical profile were uniform, and slightly lower ($S = 35$) than the salinity of the coastal sea ($S = 38$). Concentrations of reduced sulfur species (Figure 3B) were low (up to 10^{-8} M), as compared with the very high concentration (up to 10^{-4} M) recorded in April 1995.[14] Almost all detected sulfur was in the form of elemental sulfur which is in agreement with the detected oxic conditions in the Lake. Concentrations of SAS (0.15–0.18 mg/l eq. Triton-X-100) and DOC (1.5–2.2 mM) were relatively high (Figures 3C and 3D), as compared with the surrounding seawater (0.12 mg/l of SAS) and were distributed similarly to the phytoplankton cell-density along the vertical profile.

In March 1996, thermally inverted stratification (Figure 4A) could be distinguished. The upper 5 m water layer had a lower temperature and salinity (temp. 9–11 °C, $S = 25$–27) than the layer beneath 5 m (temp. 15–17 °C and $S = 35$–36). The thermocline and halocline were defined between 5 m and 7 m. Concentration of oxygen was higher in the upper layer (oxygen saturation was between 100 and 163%), which indicated the onset of primary production. Concentration of SAS ranged between 0.16–0.32 mg/l (Figure 4C). The maximum SAS value of 0.32 mg/l was recorded at the 5 m depth, where phytoplankton was most abundant, and water was oversaturated with oxygen (163%) (Figures 4A, C and D). It is well known that phytoplankton is an important source of SAS in the sea.[23,24]

Elemental sulfur was detected only in deeper waters (below 5 m) (Figure 4B), and its concentration was not essentially changed relative to October 1995. Maximum concentration of elemental sulfur (4×10^{-8} M) was determined at the 12 m depth in the hypoxic zone (concentration of oxygen was 0.12 mM), suggesting the onset of anoxic conditions. However, the possibility could be not be ruled out that at these very low concentrations the obtained sulfur peak, which we call »elemental sulfur«, belongs to the complexed sulfide as well. Namely, as reported by Luther and Tsamakis,[25] some
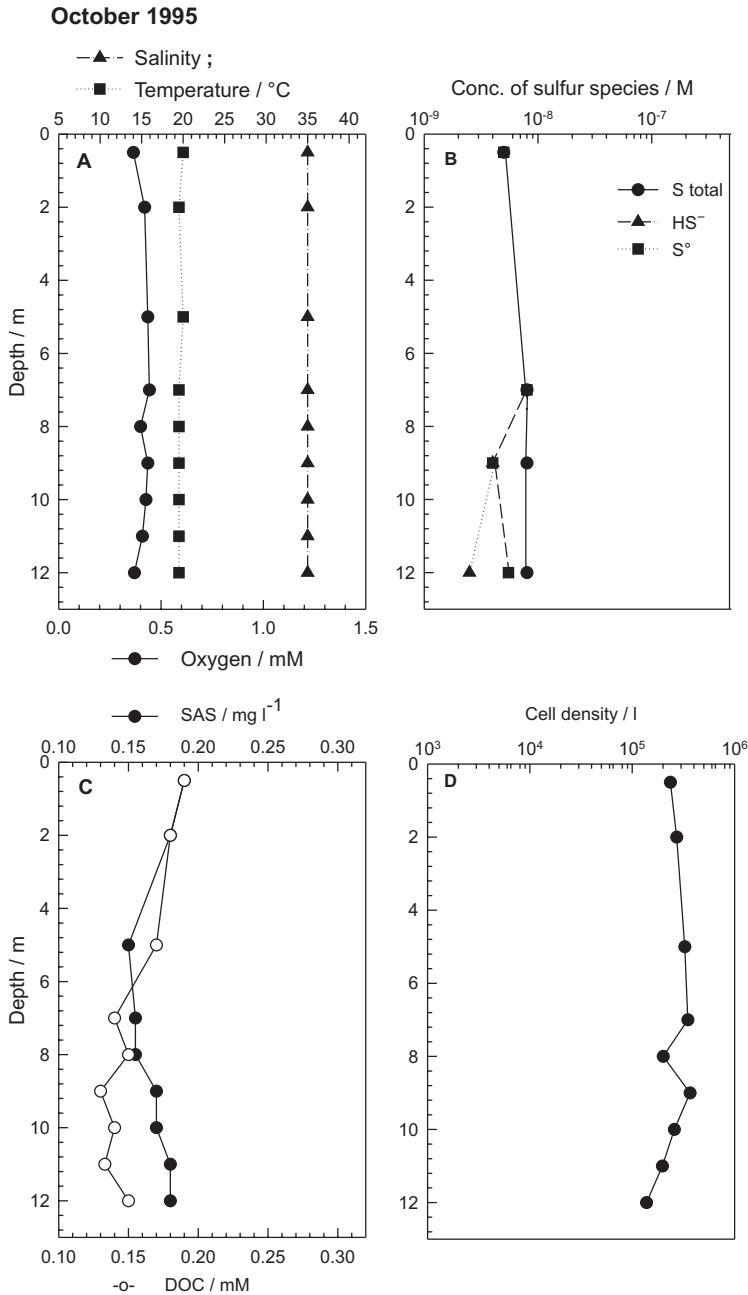
Figure 3. Vertical profiles of: (A) oxygen, temperature and salinity, (B) sulfur species, (C) surface active substances (SAS) eq. to Triton-X-100 and dissolved organic matter (DOC), (D) microphytoplankton cell-density in the Rogoznica Lake (October 27, 1995).
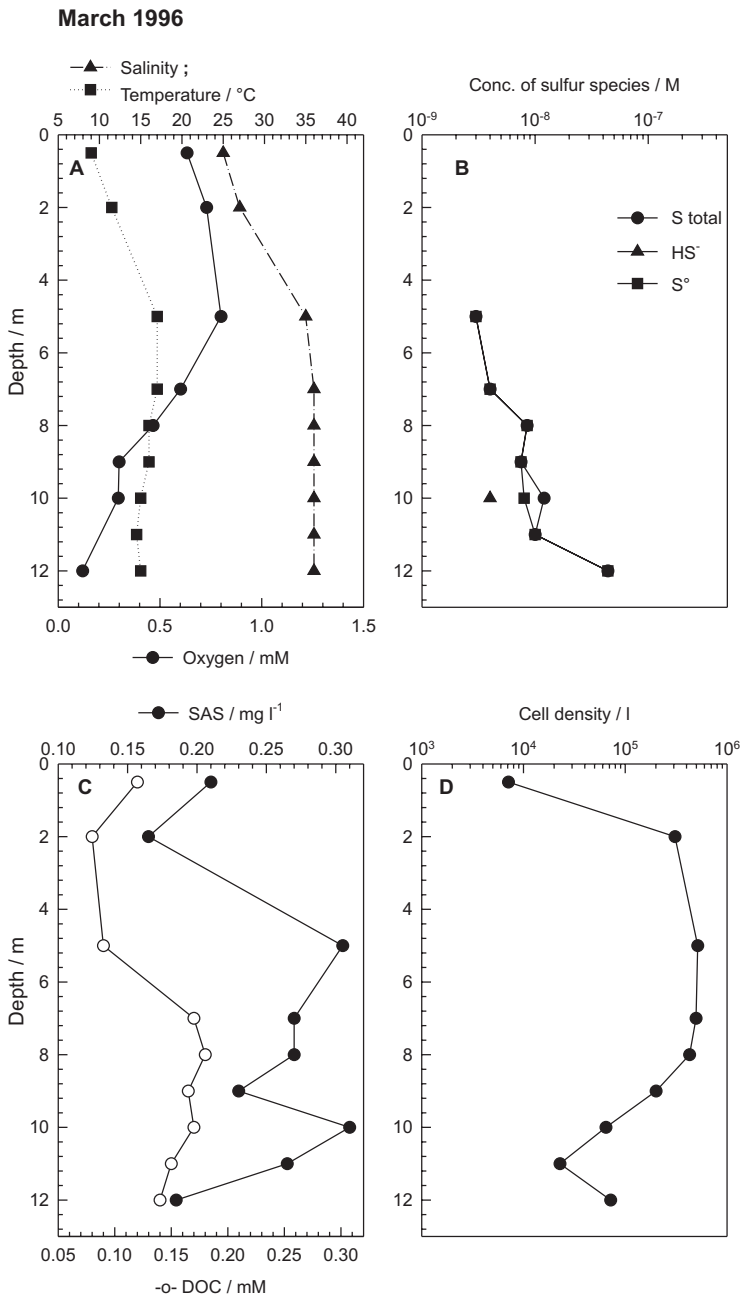
March 1996

Figure 4. Vertical profiles of: (A) oxygen, temperature and salinity, (B) sulfur species, (C) surface active substances (SAS) eq. to Triton-X-100, and dissolved organic matter (DOC), (D) microphytoplankton cell-density in the Rogoznica Lake (March 13, 1996).
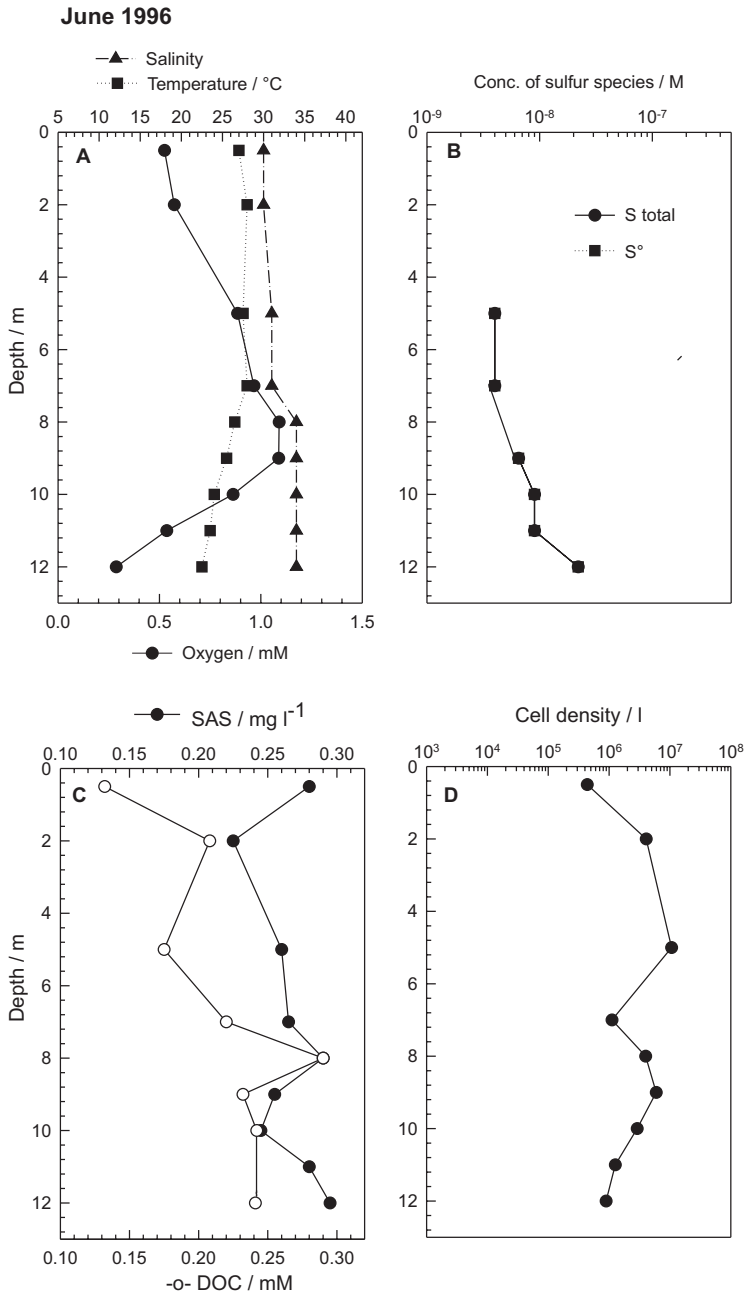
June 1996

Figure 5. Vertical profiles of: (A) oxygen, temperature and salinity, (B) sulfur species, (C) surface active substances (SAS) eq. to Triton-X-100, and dissolved organic matter (DOC), (D) microphytoplankton cell-density in the Rogoznica Lake (June 13, 1996).

of the metal sulfides are electroactive species insensitive to acidification and removal in acidic conditions.

Vertical profiles of oxygen, temperature and salinity, as well as the concentrations of sulfur, SAS, DOC and phytoplankton in the Rogoznica Lake on June 13, 1996 are shown in Figures 5 A, B, C and D. The spring bloom continued and the stratification of the Lake progressed. The upper water layer (now expanded to the depth of 8 m) was extremely warm (27–28 °C), while the salinity of the layer still remained lower ($S = 30$–31) and was influenced by spring rainfall. This influence was also visible in a decrease in the salinity ($S = 34$) in the lower layer (8–12 m), as compared to the situation recorded in March. The temperature of the lower layer was also increased and varied between 22 and 28 °C. The period of warm and sunny weather and the input of nutrient rich fresh water, initiated the outburst of diatom species *Eunotia spp.* (10 million cells/l) with a slightly expressed maximum at the 9 m depth (Figure 5D). Concentrations of SAS were high (between 0.22 and 0.30 mg/l) in the whole water column (Figure 5C), as compared to March, and the shape of obtained voltammetric curves confirmed the SAS characteristic of diatoms.[26] Concentration of oxygen was extremely high throughout the vertical profile, with maximum values at the 8 m and 9 m depths where oxygen saturation reached 270% (Figure 5A), indicating strong phytoplankton activity in deeper layers on June 13. As shown in Figures 5A, C and D, the maximum concentration of oxygen corresponded to phytoplankton accumulation (6 million cells/l) and maximum concentrations of SAS (0.29 mg/l) and DOC (0.29 mM). The vertical profile of elemental sulfur showed a similar shape as in March, although the concentration of reduced sulfur at 12 m depth was twice lower on June 13 than in March. It is believed that spring accumulation of viable diatom cells, which extended to the deeper layers of the Lake water, resulted in a raised oxygen concentration, which probably prevented anoxia and production of reduced sulfur compounds.

During the mentioned sampling periods, anoxia was not observed in the Rogoznica Lake waters above the 12 m depth, so we decided to collect the samples at the depth of 13 m (0.5 m above the bottom) expecting to find anoxic conditions there. As expected, anoxic conditions were observed below 12 m (area of chemocline), with the concentrations of total reduced sulfur at 13 m between 4×10^{-5} and 1×10^{-4} M during the summer months (June, July and August 1996) (Figure 6). The concentration of total reduced sulfur below the chemocline increased twice in the period between June and August (Figure 6) but no spreading of anoxia in upper layers was observed. All detected sulfur was mainly in the form of sulfide (84% in June and July, and 62% in August), and the rest to 100% was in the form of elemental sulfur, which is in accordance with the previously obtained data for sulfur species distribution in the Rogoznica Lake.[14] These relatively high concentrations of elemental sulfur (1–32 μM) found in the anoxic part of the Rogoznica Lake are
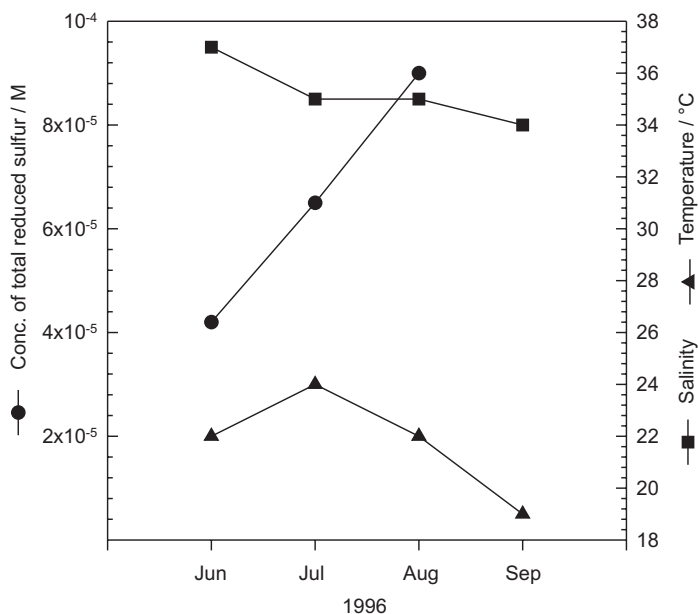
Figure 6. Variations of concentrations of the total reduced sulfur, temperature and salinity values at the 13 m depth in the Rogoznica Lake during the summer of 1996. The concentration of total reduced sulfur in September 1996 was determined at 2.2×10^{-8} M.

comparable to the values obtained in marine porewaters[18] and in the hypo-limnion of a stratified lake.[1] Also, elemental sulfur coexisting with sulfide may dissolve as polysulfides.[1] Sulfide, elemental sulfur and polysulfide have the same shape of voltammetric peaks at the same potential,[14] and so far we have not been able to distinguish electrochemically between polysulfide, free sulfur and sulfide. In the sample taken from the 13 m depth in the Rogoznica Lake in August 1996, the presence of polysulfide was detected spectrophotometrically using the method of Chen and Morris.[27] The micro-biological oxidation of sulfide in a photic zone due to the activity of purple and green sulfur bacteria can explain the abundance of particulate and dis-solved elemental sulfur.[1,11] In the sample taken on June 26, 1996, where the concentration of elemental sulfur was determined to be 7×10^{-6} M (5 times lower than the concentration of sulfide), the presence of purple sulfur bacte-ria (genus *Chromatium*) was microscopically detected. The presence of pur-ple sulfur bacteria was also indicated by a pinky colour of the water from the chemocline. In the same sample of June 26, besides the sulfide and ele-mental sulfur, the presence was also determined of thiosulfate in the con-centration of 8×10^{-6} M at the depth of 13 m, which is in accord with concen-trations of thiosulfate usually found in natural waters.[8,9] The occurrence of
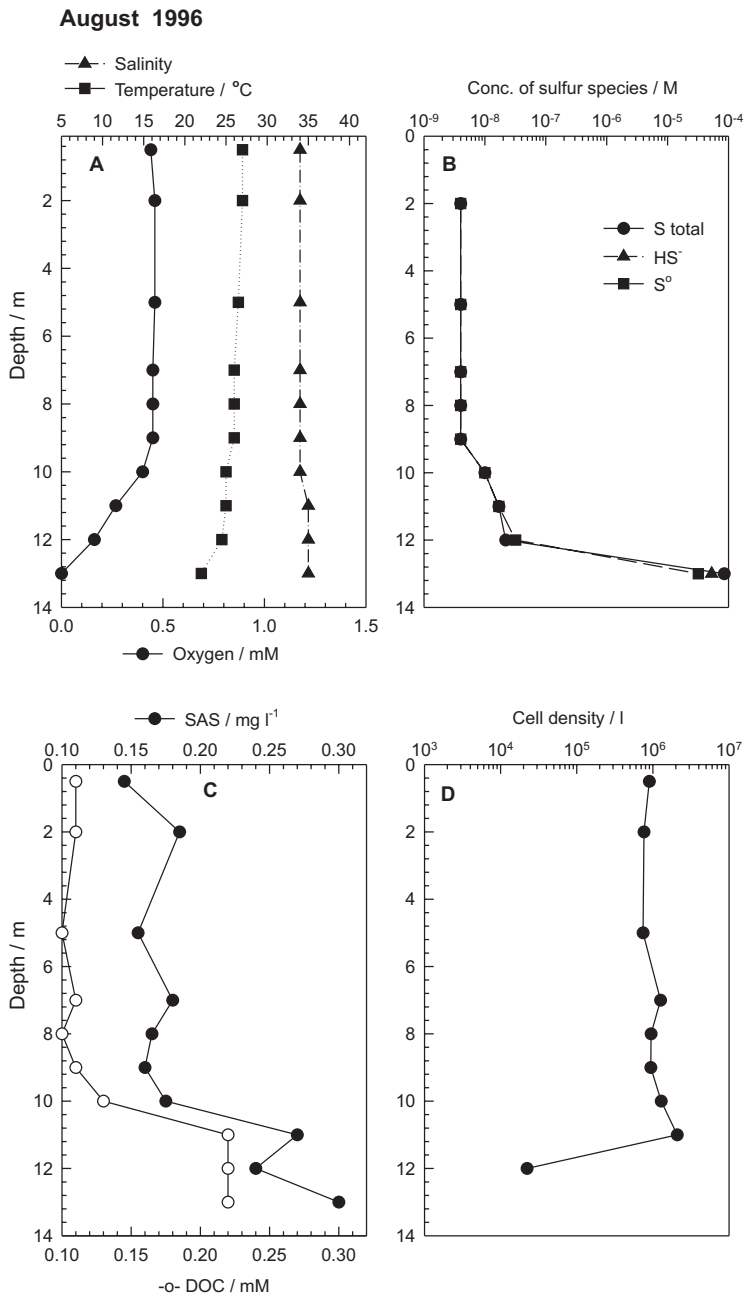
August 1996

Figure 7. Vertical profiles of: (A) oxygen, temperature and salinity, (B) sulfur species, (C) surface active substances (SAS) eq. to Triton-X-100, and dissolved organic matter (DOC), (D) microphytoplankton cell-density in the Rogoznica Lake (August 21, 1996).

thiosulfate in the chemocline is in accord with its formation by dissolved oxygen or direct photosyntetic oxidation by bacteria.[1]

Vertical profiles of oxygen, DOC and SAS values (Figure 7A and C) in August 1996 confirmed the decreased phytoplankton activity in the Rogoznica Lake. The diatom bloom had finished, and the concentrations of oxygen, DOC and SAS were decreased. In the deeper layer (depth from 10–13 m), hypoxic conditions were detected below 11 m, and anoxic conditions with high concentrations of DOC (up to 0.22 mM) and SAS (up to 0.30 mg/l) at the 13 m depth. Concentration of total reduced sulfur at the 13 m depth (Figure 7B) was also very high (up to 10^{-4} M), twice higher than in June, indicating the remineralization of organic matter produced during intensive primary production. The ratio between sulfide and elemental sulfur was determined to be 1.65 : 1, and the presence of elemental sulfur could be ascribed to the presence of polysulfide. In this sample, thiosulfate was not detected.

The end of August and the beginning of September 1996 were characterized by extremely high levels of rainfall (121.6 mm in August 1996, as compared to 44.3 mm of rain in August 1994 and 88.1 mm in August 1995). This probably caused mixing of water layers in the Lake, which resulted in sulfide oxidation. In September 1996, oxygen was detected even at the 13 m depth (concentration of oxygen was 0.17 mM), which, along with the decreased values of salinity (S = 34) and temperature (19 °C), pointed to the strong mixing of lake water.

Concentration of total sulfur at the 13 m depth decreased in September to 2.2×10^{-8} M. No sulfide was found in the Lake to the depth of 13 m, and all reduced sulfur was present in the form of elemental sulfur, which is in good correlation with the detected hypoxic conditions.

Processes of water exchange between the Lake and the seawater surrounding the peninsula are still not well known and we could only suppose that water mixing that occurred in September was caused by the high level of rainfall. Since the Lake is sheltered by steep cliffs, there is practically no wind effect on water mixing. Consequently, it could be guessed that probably both possibilities, increased input of surface fresh water (rain) and subterranean input of fresh groundwater, led to overturn.

CONCLUSIONS

According to the results shown above, it might be concluded that Rogoznica Lake is a small, intermittently anoxic system, where the time scale of the anoxic water renewal varies by the order of weeks, months or years. In such systems, anoxic conditions appear mostly in summer months as the result of increased stratification of the water column in spring, with conse-

quent curtailment of reoxygenation of the bottom waters across the halocline and benthic decay of organic detritus accumulated from plankton blooms which occurred during the previous spring and summer. Moreover, Rogoznica Lake represents a unique model system in the Adriatic Sea for studying natural eutrophication processes, as distinguished from different parts of the Mediterranean Sea as well as in the northern Adriatic region with registered anthropogenic eutrophication.[28]

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SAŽETAK

Sezonske promjene anoksičnih uvjeta u Rogozničkom jezeru

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Sezonske promjene temperature, saliniteta, koncentracije kisika te okomite raspodjele otopljenog organskog ugljika, površinski aktivnih tvari, fitoplanktona i reduciranih oblika sumporovih spojeva, praćene su u malom (najveća dubina 15 m), morskom, eutroficiranom jezeru (Rogozničko jezero) tijekom 1994., 1995. i 1996. godine. Anoksični uvjeti u jezeru, s visokom koncentracijom sumporovih spojeva (do 10^{-4} M, u obliku sulfida) pronađeni su na dubinama ispod 9 m u vrijeme izražene stratifikacije tijekom 1994. i 1995. godine. Na granici između oksičnih i anoksičnih uvjeta (kemoklina) određene su fotosintetske sumporne bakterije (vrsta *Chromatium*), te prisutnost tiosulfata u koncentraciji od 8×10^{-6} M. Miješanje vode u jezeru uzrokuju padaline što pokazuje sniženje saliniteta u dubljim slojevima. Visoka fitoplanktonska aktivnost duž cijelog vodenog stupca kao i neuobičajena visoka proizvodnja kisika (zasićenje kisikom do 300%) vjerojatno je spriječila razvoj anoksije u proljeće 1996.