Acta Adriatica 66 (2025):
https://doi.org/10.32582/aa.66.1.1

www.izor.hr

ORIGINAL ARTICLE

Reduced sulfur species in a marine euxinic environment of Rogoznica Lake – Dragon Eye, Croatia

Sarah Mateša[1], Iva Dominović[1], Marija Marguš[1] and Irena Ciglenečki[1*]

[1]Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia

Abstract: Rogoznica Lake (RL) – Dragon Eye is a unique, eutrophic marine lake on the eastern Adriatic coast. The lake exhibits seasonal variations between stratified and anoxic holomictic conditions. Under stratified conditions, RL shows typical euxinic features characterized by an euxinic hypolimnion (< 8 meters deep in recent years) with a relatively high total concentration of reduced sulfur species (RSS_{tot}). These species are mainly present in the form of sulfides, non-volatile RSS (RSS_{nv}) attributed to elemental sulfur (S^0), and polysulfides (S_x^{2-}). The concentration of RSS_{tot}, particularly the RSS_{nv} and S_x^{2-}, varies seasonally depending on the physicochemical conditions in the water column, i.e. the position and stability of the chemocline and the entire water column, expressed by the Schmidt Stability Index (SSI), as well as the light intensity reaching this layer. In the summer months, when stratification is the strongest, the concentrations of the RSS_{nv} in and below the chemocline are generally higher. In addition, the highest concentrations of S_x^{2-} (up to 90 µM of polysulfidic S^0) are detected during this period. This increase is related to a more intense microbial activity of phototrophic purple sulfur bacteria (PSB), which then reach maximum abundance due to the increased temperature and maximum light intensity. Typically, the highest concentrations of S_x^{2-} are found below the chemocline. Long-term data indicate that the concentration of RSS_{nv} at the chemocline and 1 m below it depends on the hydrostatic stability of the water column and shows periodic changes. This contrasts with the previously recorded trend of increasing RSS_{tot} in the anoxic monimolimnion.
Keywords: reduced sulfur species; elemental sulfur; polysulfides; long-term data; marine euxinic lake

Sažetak: REDUCIRANE VRSTE SUMPORA U MORSKOM EUKSINSKOM OKOLIŠU ROGOZNIČKOG JEZERA – ZMAJEVOG OKA, HRVATSKA. Rogozničko jezero (RJ) - Zmajevo oko je jedinstveno, eutrofno morsko jezero na istočnoj obali Jadrana koje sezonski varira između stratificiranih i holomiktičnih anoksičnih uvjeta. U stratificiranim uvjetima, jezero ima tipična euksinska svojstva kada je donji sloj (u zadnje vrijeme obično < 8 m dubine) karakteriziran relativno visokom koncentracijom ukupnih reduciranih sumpornih specija (RSS_{tot}) i to uglavnom u obliku sulfida, nehlapljive frakcije RSS_{nv}, koja se pripisuje prisutnosti elementarnog sumpora (S^0) i polisulfida (S_x^{2-}). RSS_{tot}, posebno RSS_{nv} i S_x^{2-}, variraju sezonski, ovisno o fizičko-kemijskim uvjetima unutar vodenog stupca, tj. položaju kemokline, njezinoj stabilnosti te stabilnosti cijelog vodenog stupca (izraženog pomoću Schmidtovog indeksa stabilnosti, SSI), kao i intenzitetu svjetlosti koji dopire do kemokline. Tijekom najizraženije stratifikacije u ljetnim mjesecima, koncentracije RSS_{nv} u kemoklini i ispod nje obično su više. Također, u ljetnim mjesecima pri višim temperaturama, kada je i najveći intenzitet svjetlosti što doprinosi intenzivnoj mikrobiološkoj aktivnosti fototrofnih ljubičastih sumpornih bakterija (PSB), utvrđena je i prisutnost najviših koncentracija S_x^{2-} (do 90 µM polisulfidnog S^0) s maksimumom ispod kemokline. Dugoročni podaci RSS_{nv} u sloju kemokline i 1 m dublje, pokazuju ovisnost o stabilnosti vodenog stupca, ukazujući na periodične promjene, za razliku od koncentracija RSS_{tot} za koje je ranije pokazan porast u monimolimnionu na dugoročnoj skali.
Ključne riječi: reducirane sumporne specije; elementarni sumpor; polisulfidi; dugoročni podaci; morsko euksinsko jezero

INTRODUCTION

Sulfate reduction is a crucial biochemical process that regulates the redox state of seawater. It produces volatile sulfides (mainly as HS^-), which dominate the concentration of reduced sulfur species (RSS) in euxinic environments (Jørgensen *et al*., 1982; Bura-Nakić *et al.*, 2009; Mateša *et al*., 2024). Other RSS, such as elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), and sulfite (SO_3^{2-}), act as intermediates in the biogeochemical sulfur cycle (Helz, 2014). Dissolved elemental sulfur (S_8(aq)), a non-volatile RSS fraction, typically occurs in low concentrations due to its hydrophobic nature and low solubility. It can be incorporated into organic matter (OM) or transformed into colloidal sulfur (S_8(s)) based on water column conditions (Bura-Nakić *et al.*, 2009).

An euxinic environment, characterized by oxygen-free conditions with free hydrogen sulfide in the water column, can form under highly stratified conditions. This type of marine environment, often referred to as the Canfield Ocean (Meyer and Kump, 2008), typically consists of an upper oxic layer with intense primary production and a lower anoxic layer rich in sulfur species (Jørgensen *et al.*, 1979; Ciglenečki *et al.*, 2005; 2017 and references therein). A layer with distinct properties can develop at the boundary between these layers,

**Corresponding author:* irena@irb.hr
Received: 24 October 2024, accepted: 17 January 2025
ISSN: 0001-5113, eISSN: 1846-0453

marked by abrupt changes in physicochemical, redox, and biochemical parameters (Zadereev *et al.*, 2017). The position and thickness of the chemocline can vary with environmental conditions and influence the distribution and dynamics of RSS (Avetisyan *et al*., 2019).

In the chemocline, high biochemical and microbial activity drives numerous oxidation-reduction processes. Partially reduced sulfur compounds formed by sulfate reduction can be converted to more stable oxidation states such as sulfide, elemental sulfur, and sulfate (Jørgensen *et al.*, 1979; Barbash and Reinhard, 1989; Overmann *et al.*, 1996). Changes in pH and redox conditions directly influence sulfur speciation in the chemocline, while temperature, light intensity, and microbial activity exert indirect effects (Stumm and Morgan, 1996).

Chemoautotrophic and photoautotrophic microorganisms in the chemocline oxidize sulfide to biological sulfur (S^0), which is hydrophilic due to adsorbed organic polymers, and thus more soluble than inorganic sulfur (Kleinjan *et al.*, 2003). Under anoxic conditions, sulfur is present in forms such as inorganic polysulfides (S_x^{2-}), polythionates, organic polysulfides, and organic polysulfanes (Helz, 2014). S_x^{2-}, along with HS^-, accounts for most of RSS_{tot} in euxinic waters, with zero-valent sulfur from S_x^{2-}contributing to RSS_{nv} (Bura-Nakić *et al.*, 2009; Mateša *et al.*, 2024).

Polysulfides (S_x^{2-}) play a crucial role in biogeochemical processes: they buffer zero-valent sulfur activity, form pyrite, transform OM in sediments, and form strong complexes with metals (Helz *et al.*, 2014). S_x^{2-} can be formed by sulfide oxidation, the reaction between sulfide and elemental sulfur, or the reduction of elemental sulfur (Kleinjan *et al.*, 2003). In aqueous sulfide-containing solutions, S^0 dissolves into S_x^{2-} (Giggenbach, 1972; Boulegue, 1978; Kleinjan *et al.*, 2003; Kamyshny *et al.*, 2004; Helz, 2014). Biological sulfur is a crucial source for S_x^{2-} formation (Kleinjan *et al.*, 2003, 2005; Findlay, 2016). In addition, layers of bacteria of the *Chromatiaceae* family protect *Chlorobiaceae* from O_2, to which they have almost no tolerance. In return, they can provide extracellular elemental sulfur that reacts abiotically with sulfides to form polysulfides, which may be used immediately by members of the *Chromatiaceae* family (Kushkevych *et al*., 2021).

Electroanalytical methods (EM) are widely used to monitor RSS in natural waters, including volatile (mainly HS^-) and non-volatile RSS (mainly elemental S^0, and polysulfidic S^0) (Ciglenečki *et al*., 2014). Differential pulse voltammetry (DPV) is effective for the detection of polysulfidic sulfur in highly concentrated electrolyte solutions (Kariuki *et al.*, 2001). Recently, Mateša *et al.* (2024) applied this method for the detection of polysulfidic sulfur in the euxinic marine environment of Rogoznica Lake (RL) on the Adriatic coast. The RL can be considered as a natural laboratory for studying RSS biogeochemical cycles and redox chemistry in stratified seawater column (Helz *et al.*, 2011; Kamyshny *et al.*, 2011; Ciglenečki *et al.*, 2017, and references therein).

RL is characterized by the perennial thermohaline stratification (Fig. 1A), which promotes chemical stratification that allows permanent anoxia and RSS accumulation in its shallow euxinic monimolimnion (below 8 meters depth, maximum depth 14 meters) (Čanković *et al.*, 2019; Dominović *et al.*, 2023). The chemocline depth has become shallower since 1996, with increasing volumes of deoxygenated water enriched with RSS (Dominović *et al.*, 2023) and organic matter (Simonović *et al.*, 2023). Long-term trends show increasing temperatures (Čanković *et al.*, 2019) and decreasing salinity (Mifka *et al.*, 2022; Dominović *et al.*, 2023), which affects water column stability and the stratification intensity (Ciglenečki *et al.*, 2015; Dominović *et al.*, 2023).

In recent years, abrupt breaks in stratification have occurred frequently (1997, 2011, 2016, 2020, 2021), leading to anoxic holomictic conditions throughout the water column (Fig. 1B) and impacting the biology and biogeochemistry of the RL (Kršinić *et al*., 2000; Ciglenečki *et al*., 2017; Marguš *et al*., 2023). These unique characteristics of RL provide an opportunity to study RSS speciation and dynamics using EM (Ciglenečki *et al.*, 1996, 2014; Ciglenečki and Ćosović, 1997; Bura-Nakić *et al.*, 2009; Mateša *et al.*, 2024). Therefore research focus of this paper lays on the discussion of long-term data on RSS_{nv}, mainly attributed to S^0 formed by polysulfide decomposition (Bura-Nakić *et al*., 2009), and recent direct measurements of polysulfidic S^0 equivalents of S_x^{2-} (Mateša *et al*., 2024). The RSS_{nv} were discussed as a function of different physicochemical parameters measured in the RL water column (temperature, salinity, dissolved organic carbon (DOC), particulate organic carbon (POC), surface-active substances (SAS), RSS_{tot} and the hydrostatic stability of the lake water column). Exploring the dynamics of RSS in RL enhances our understanding of sulfur biogeochemistry in euxinic environments, offering valuable insights into redox processes, microbial activity, and environmental transformations within stratified aquatic systems, including marine ecosystems, which are anticipated to expand further in response to climate change.

MATERIALS AND METHODS

Study site

Rogoznica Lake (RL) (Fig.1), also known as Dragon Eye (43°32’ N, 15°58’ E), is a small marine lake located on the central Croatian coast of the Adriatic Sea, with a surface area of 9,904 m² (Panđa, 2020). This unique euxinic environment is characterized by alternating anoxic holomictic and meromictic conditions (Ciglenečki et al., 2017). The strong and stable stratification of the water column, based on different densities and chemical properties of the water layers, is one of the most important characteristics of this lake. Most of the time, the water column is stratified into an upper oxic layer and a lower anoxic layer rich in RSS, nutrients and organic

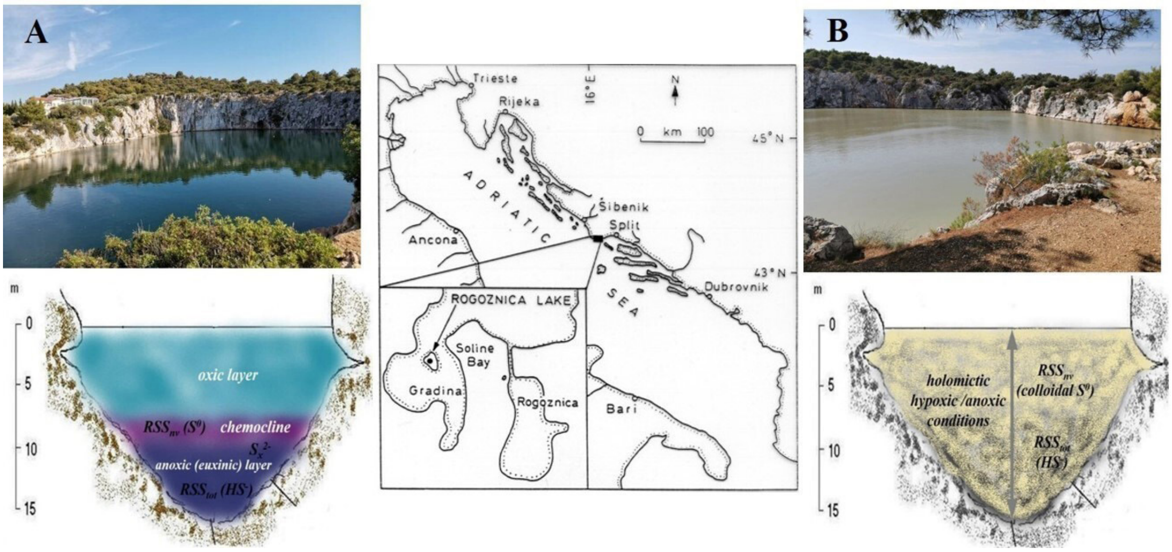

Fig. 1. A map of Rogoznica Lake (middle panel) accompanied by photos and vertical profiles of the lake's water column displayed on the left and right. These illustrate: the chemocline and the euxinic layer enriched with RSS during stratified conditions in spring **(A)** and the holomictic mixing of the water column in autumn **(B)**.

matter (Ciglenečki *et al.*, 2015) (Fig. 1A). A pink-colored chemocline (thickness up to 50 cm) forms seasonally at the boundary between oxic and anoxic layers. The chemocline is characterized by a dense population of purple phototrophic sulfur bacteria, PSB, and, in some cases, even a large abundance of green sulfur bacteria (GSB) was confirmed (Pjevac *et al.*, 2015; Čanković *et al.*, 2017, 2019), as the main biological source of S. Under euxinic conditions, this S^0 of biological origin can be dissolved by HS^- into nucleophilic polysulfides, S_x^{2-} (Kleinjan *et al.*, 2003, 2005; Findlay, 2016). The stratification and mixing of the water layers are directly influenced by meteorological conditions, especially the surface heat flux, air temperature, rain, surface runoff (including tidally driven exchange of water through porous karst) and strong wind (Ciglenečki *et al.*, 2015; Dominović *et al.*, 2023, 2024). The small size and physically stable nature of the lake water column allow research into spatial and temporal variability on a small scale as well as long-term processes (Ciglenečki *et al.*, 2006, 2017).

A wide concentration range of RSS (nM – mM) can be found in the RL water column including organic and inorganic dissolved and colloidal sulfur species, making this environment an ideal location for testing and developing methods to characterize and specify different RSS (Bura-Nakić *et al.*, 2009; Ciglenečki *et al.*, 2014; Mateša *et al.*, 2024). At the same time, the lake provides a great opportunity to study the microbial diversity that controls the biogeochemical cycling of sulfur (Čanković *et al.*, 2017, 2019, 2020).

Sampling and analyses

During sampling, physico-chemical parameters, temperature (T), dissolved oxygen (O_2) and salinity (S) were measured *in situ* with a CTD probe from the surface to the bottom (12 m). In addition, T, S, O_2, and light intensity automatic data loggers have been positioned in different layers of the RL water column from 2019 to 2021, with a data acquisition frequency of one hour.

Samples of the RL water column have been taken at different seasons of the year since 1996. Sampling was always carried out at the same location at the central position in RL (Fig. 1) with Niskin bottles (5 L) or by a diver at depths of 0, 2, 5, 7, 8, 9, 10 and 12 meters. Samples for RSS analysis were always taken first to prevent oxidation and to maintain anoxic conditions in the samples.

The RSS concentration was determined by electrochemical methods using a three-electrode system of the automated 663 VA Stand Metrohm electrode with mercury (Hg) as the working electrode, an Ag/AgCl (3M KCl) as a reference and a graphite rod as a counter electrode. The measurements were carried out in a deoxygenated electrochemical cell (purged with N_2), in undiluted samples from the oxic layer and, due to the high RSS concentration, in diluted samples from the anoxic layer as already described (Ciglenečki *et al.*, 1996, 2005, 2014; Marguš *et al.*, 2015; Bura-Nakić *et al.*, 2009).

The total concentration of RSS (RSS_{tot}) was determined by linear sweep (LSV) and/or by cathodic stripping voltammetry (CSV) from the initial deposition potential E_{dep} = -0.2 V (*vs.* Ag/AgCl) by negative scanning to the final potential E_p = -1.6 V with an accumulation time t_a = 0 - 120 s (Ciglenečki *et al.*, 1996, 1998, 2014; Ciglenečki and Ćosović, 1997; Bura-Nakić *et al*., 2009; Marguš *et al*., 2015). The non-volatile fraction of RSS (RSS_{nv}) was determined by adding HCl to the sample to achieve a pH of about 2 and flushing out the volatile fraction by flowing N_2 into the sample in the cell. After the pH was brought back to the original value with

NaOH, the measurements were repeated and the results obtained in this way corresponded to the RSS_{nv} concentration, which was attributed to the presence of S^0, including polysulfidic S^0 (Mateša *et al*., 2024).

Polysulfidic sulfur ($S^0_{polysulfidic}$) equvivalent of S_x^{2-} has been determined by differential pulse voltammetry (DPV) without accumulation in the potential range from E = - 0.8 V to -1.2 V in recently studied samples since 2019 as described in detail in Mateša *et al.* (2024).

Organic matter (OM) concentrations, represented as DOC and POC, were determined using sensitive high-temperature catalytic oxidation (HTCO) as previously described (Dautović *et al.,* 2012, 2017). The surface active fraction of OM (surface active substances, SAS) as the most reactive fraction of DOM, and DOC representing different OM hydrophobicity, was determined by a.c. voltammetry (Ćosović and Vojvodić, 1982, 1998; Ciglenečki *et al.*, 2023).

The hydrostatic stability of the water column can be expressed by the Schmidt Stability Index (SSI; J m^{-2}), which indicates the energy required to bring the water column from a stratified to a uniform state (Idso, 1973). The SSI values were calculated in the same manner as in the work of Dominović *et al.* (2023).

Correlation analyses were performed in Origin 8 software (OriginLab) using the Pearson correlation method, which tells whether relationships are statistically significant (depending on the p-value; in this study, we used $p < 0.05$), and provides information on the strength of such relationships (correlation) with the Pearson coefficient, r, whose range can be between -1 (indicating a perfect negative correlation) and +1 (indicating a perfect positive correlation). The strength of the correlation is expressed as a function of r: for a very weak correlation, r is from ±0.25 to ± 0.50; a good correlation means r is from ±0.50 to ±0.75; and a very good and excellent correlation is indicated by r being from ±0.70 to ± 1.00 (Udovičić *et al.*, 2007).

RESULTS

Temporal and spatial dynamics of RSS in the RL water column as a function of physicochemical parameters

The vertical distribution of physico-chemical parameters such as T, S, density (σ_T), light intensity, and dissolved O_2 concentration, which influence the stability of stratification and the occurrence of holomixia, and have a strong impact on RSS concentration and speciation, is shown in Fig. 2 for different water column conditions: stable stratification in May 2019; weak stratification in July 2020; and anoxic holomictic conditions in October 2020. In addition to these basic factors, other parameters, including OM components of DOC and POC are plotted alongside RSS and polysulfide concentrations. Measured values for RSS including RSS_{nv} and polysulfidic S^0 are given in Table 1.

In May 2019, the surface temperature of RL was 20.7 °C, while the temperature below the surface, at a depth from 2 to 6 m, was higher (23 °C). At the chemocline, the temperature dropped to 17 °C (Fig. 2A, yellow full circles). This temperature trend corresponds to a change in salinity, with the halocline at 10 m depth (Fig. 2A, yellow circles). Consequently, σ_T differences between the 0-9 m and 10-12 m layers ($\Delta\sigma_T$ = 2.55 kg/m^3) are evident, with the highest σ_T value of 22.97 kg/m^3 at 10 m depth (Fig. 2B, yellow circles). The oxygen concentration varied from 9.22 mg/L at the surface to a maximum value of 21.5 mg/L at 7 m depth. At the chemocline, between 8 and 9 m depth, the oxygen concentration dropped to 0 mg/L, leading to hypoxic and anoxic conditions at greater depths (Fig. 2B, yellow full circles). As a result, the total RSS concentration increased from 8 m to 12 m, with a peak concentration of 700 µM as shown in Fig. 2C (yellow full circles). Below 9 m, polysulfides were detected with a maximum concentration of 60 µM $S^0_{polysulfidic}$ at 11 m depth (Fig. 2C, yellow circles). During this period, the DOC was highest at the surface (3.83 mg/L) as shown in Fig. 2D with yellow full circles, while the POC was lowest at 2 m depth (0.40 mg/L) and increased with depth, reaching a maximum of 3.29 mg/L at 10 m depth before decreasing (Fig. 2D, yellow circles).

In July 2020, the surface temperature of RL was 27.3 °C, and gradually decreased to 21 °C at 10 m depth (Fig. 2A, cyan full triangles). Salinity remained fairly constant (35.2 ± 0.3) as shown in Fig. 2A (cyan triangles). Density differences between layers (Fig. 2B, cyan triangles) were lower than in 2019 ($\Delta\sigma_T$ = 1.90 kg/m3), indicating reduced stability of the RL water column, with higher σ_T at 12 m (σ_T = 25.4 kg/m³) compared to 2019. Oxygen concentrations were the highest in the surface layer (9.40 ± 0.2 mg/L) but dropped to hypoxic values at 7 m (2 mg/L) and anoxic values at 8 m (~0 mg/L) as shown in Fig. 2B (cyan full triangles). RSS_{tot} concentrations increased from 9 m to 12 m and peaked at 2130 µM (Fig. 2C, cyan full triangles). In contrast to 2019, polysulfides were detected at very low concentrations (1 - 6 µM $S^0_{polysulfidic}$) in July 2020 (Fig. 2C, cyan triangles). The DOC was highest at the bottom of the lake (2.46 mg/L) as shown in Fig. 2D (cyan full triangles) and the POC was maximal above the chemocline at 7 m (0.77 mg/L) as shown in Fig. 2D with cyan triangles, which was higher than the value at the same depth in May 2019 (POC = 0.62 mg/L), suggesting increased turbidity and reduced light penetration in the deeper layers.

In October 2020, the surface temperature of RL was the lowest (19 °C), with relatively uniform temperatures of ~ 21 °C from 2 m depth to the bottom at 12 m (Fig. 2A, black full squares). Salinity and density were relatively uniform throughout the water column as well (35.5 ± 0.5 in Fig. 2A and 25 ± 0.6 kg/m³ in Fig. 2B, respectively; both marked with black squares). Except for the surface layer, where the oxygen concentration was high (17 mg/L), indicating strong primary production, the rest of

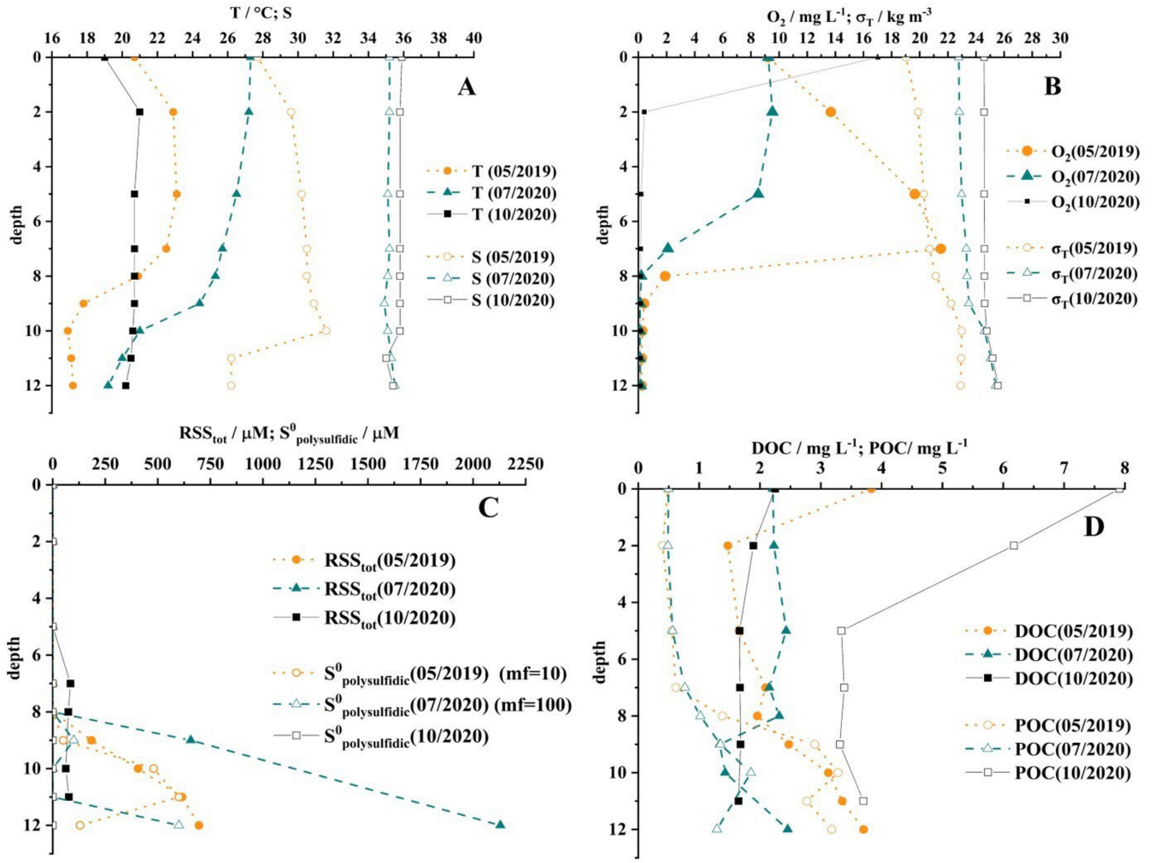

Fig. 2. Vertical distribution of physicochemical parameters in the RL water column: T and S **(A)**, dissolved O_2 and σ_T **(B)**, total RSS and $S^0_{polysulfidic}$ **(C)**, DOC and POC **(D)** in different seasons - spring (May 30, 2019) (yellow lines with circles); summer (July 28, 2020) (cyan lines with triangles) and autumn (October 20, 2020) (black lines with squares). (mf = multiplication factor – $S^0_{polysulfidic}$)

Table 1. Reduced sulfur species (RSS) concentrations in the Rogoznica Lake water column.

Sampling date	Chemocline depth / m	sample	c (RSS_{tot} eq HS^-) / µM	c ($S^0_{polysulfidic}$)/ µM	c (RSS_{nv} eq HS^-) / µM
30.05.2019.	9	C	184	5	n.m.
		BC	406	48	n.m.
		A	655	36.5	n.m.
01.07.2019.	8.7	C	173	70	n.m.
		BC	356	89	n.m.
27.08.2019.	9	C	376	18	170
		BC	485	4	218
		A	509	49	n.m.
17.07.2019.	8	C	1400	23	1600
21.01.2020.	5	A	344	4	7
28.06.2020.	7.7	C	468	21	207
		BC	330	27	260
		$BC_{afternoon}$	892	41	172
28.07.2020.	8	BC	657	1	116
		A	2130	6	95

C = chemocline; BC = 1 m below chemocline; A = anoxic layer; n.m. = not measured the water column was completely depleted in terms of oxygen content (0 mg/L, from 2 to 12 m) (Fig. 2B, full black squares). Total RSS concentration ranged from 5 nM at the surface to 60 - 85 μM in the 5 to 12 m layer (Fig. 2C, full black squares). In contrast to the stable and reduced stratification conditions (May 2019 and July 2020, respectively), S_x^{2-} was not detected under mixing conditions in the water column (Fig. 2C black squares). The values of DOC (2.25 mg/L) (Fig. 2D, full black squares) and POC (7.91 mg/L) (Fig. 2D, black squares) were the highest at the surface and decreased with depth (DOC = 1.68 mg/L; POC = 3.14 mg/L).

The observed results highlight differences in the distribution and concentrations of S_x^{2-} under various conditions in the RL water column. Consequently, the stability of the water column and the light intensity at the chemocline, both of which may have a direct influence on the microbial activity of phototrophic bacteria (e.g., the occurrence of PSB and GSB), were further analyzed. The dependence of the polysulfide concentration in the chemocline and below on light intensity at the chemocline and the SSI of the water column is illustrated in Fig. 3.

Primarily focusing on RL samples from the summer season, Fig. 3 highlights the period when the highest concentrations of S_x^{2-} were measured. In general, the highest polysulfidic S^0 concentrations were found in samples taken below the chemocline. The highest concentrations (89 μM) were measured when the light intensity reaching the chemocline was also the highest (about 3500 lux) on July 1, 2019. Lower concentrations were observed when light intensity was lower (05/2019 and 08/2019) (Table 1) or did not reach the chemocline layer (summer 2020). The temperature distribution in the RL water column shows a maximum in the layer below the surface (0-5 m), with an average of 22.2 °C in May 2019 and 27.0 °C in July 2020. Most radiation and light is absorbed in this layer, as indicated by the data on light intensity in 2019 when the temperature maximum was lower.

The results in Fig. 3 indicate a direct influence of water column stability (expressed by the SSI) on S_x^{2-} concentrations. When the SSI was highest in winter (> 600 J m⁻²), light intensity and water column T were relatively low (15 °C at the chemocline), consequently indicating lower microbial activity at the chemocline (Čanković, 2018; Čanković *et al.*, 2017, 2019, 2020). In winter, the polysulfides were measured in very low concentrations (4 μM $S^0_{polysulfidic}$). In the summer months,

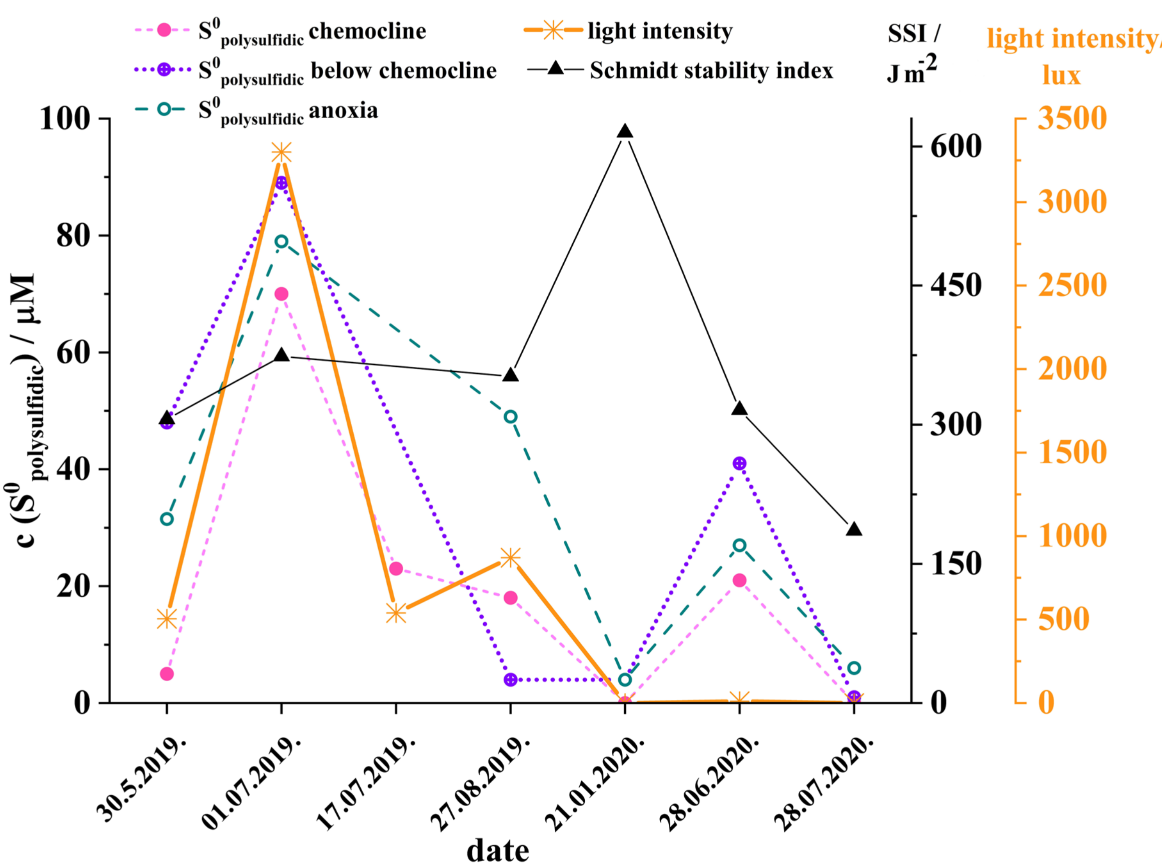

Fig. 3. $S^0_{polysulfidic}$ concentration dependence on the stability of the water column (Schmidt stability index, SSI, black line with triangles) and light intensity (orange line with stars) for RL samples from different seasons of 2019 and 2020 in the chemocline layer (pink line), below the chemocline layer (purple line) and in the anoxic layer at 12 m depth (cyan line).

the higher S_x^{2-} concentrations were also observed with more pronounced stability of the water column (with higher SSI), increased light intensity, and higher T in the chemocline zone. Light intensity, T, and SSI values also reflect differences in OM content (total organic carbon, TOC = DOC + POC) in the layer above the chemocline. In May 2019, TOC above the chemocline averaged 2.41 mg/L; in August 2019, it was 1.98 mg/L; and in July 2020, when light intensity and S_x^{2-} concentration were the lowest, TOC was the highest at 2.83 mg/L.

Long-term data on RSS_{nv} concentration in the RL

To understand the dynamics of RSS_{nv} in the RL water column, particularly S_x^{2-}, which was not directly measured in the RL water column before 2019, but is assumed to contribute largely to RSS_{nv} through the decomposition of S_x^{2-} (Bura-Nakić *et al.*, 2009; Mateša *et al.*, 2024), the long-term data (1996-2021) on RSS_{nv} concentration were analyzed. The long-term trends of the non-volatile fraction of RSS in two regions, i.e. the chemocline (represented by the purple-pink line with circles) and the anoxic layer 1 meter below the chemocline, where higher concentrations of S_x were typically measured (shown as a black-purple line with squares) are presented in Fig. 4. Additionally, the figure includes the corresponding water column stability, indicated by the SSI as a black line with triangles (Dominović *et al*., 2023).

The results in Fig. 4 reveal periodic variations in RSS_{nv}:

1. Period 1996-2000: RSS_{nv} concentrations reached up to 150 µM, with anoxic conditions confined to water layers below 10 meters.
2. Period 2006-2010**:** higher RSS_{nv} concentrations, up to 400 µM, were observed with the chemocline positioned at a depth of 9 meters. During this period, there were two cases of partial water column mixing in late autumn (in 2006 and 2009) as noted by Ciglenečki *et al.* (2015).
3. Period 2017-2020: RSS_{nv} concentrations up to 200 µM were detected, with the chemocline at a depth of 8 meters. This period coincided with water column overturns recorded in 2016 and 2020 (Čanković *et al.*, 2020; Simonović *et al.*, 2023).

The stability of the RL water column (represented by the black line with triangles) influences the RSS_{nv} concentrations, as shown in Fig. 4. Generally, RSS_{nv} values are the lowest (< 20 µM) during periods of lower stability, as indicated by SSI values lower than 500 J m^{-2} (e.g., 2008; 2011-2015). It is interesting to note that similar trends have been observed in the long-term monitoring of DOC dynamics (Simonović *et al.*, 2023).

Given the lack of clear long-term trends in RSS_{nv} concentration, or during specific periods and seasons, a statistical analysis was conducted to explore correlations between RSS_{nv} concentration and other physicochemical parameters. Table 2 presents the results of linear regression analyses, including the long-term correlations (1996-2021), correlations by season (spring, summer, fall, winter), and correlation in specific periods (1996-

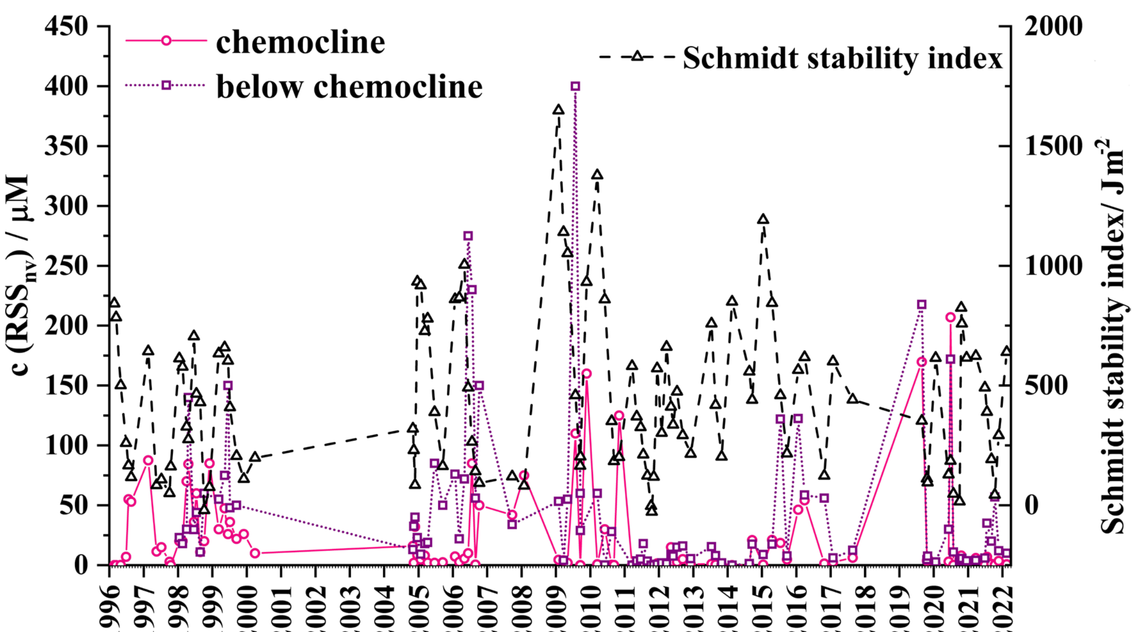

Fig. 4. Long-term RSS_{nv} concentration in the RL chemocline layer (pink line with circles) and 1 m below the chemocline layer (purple line with squares) in relation to the corresponding Schmidt stability index, SSI (black line with triangles) for the period 1996-2021.

Table 2. Correlations of non-volatile reduced sulfur species (RSS_{nv}) and physicochemical parameters in the chemocline and 1 m below the chemocline in RL on a long-term scale (1996-2021) and in the selected periods: 1996-2007; 2008-2013; 2014-2020; statistically significant correlations ($p < 0.05$) are marked with *.

	RSS_{nv} chemocline						
	T	S	DOC	POC	SAS	RSS_{tot}	SSI
1996 - 2021	-0.13	0.16	0.29*	0.06	0.29*	0.46*	-0.09
1996 - 2007	0.01	-0.05	0.19	n.m.	0.34	0.48*	-0.25
2008 - 2013	-0.20	0.17	0.34*	-0.09	0.41*	0.49*	0.06
2014 - 2020	0.16	0.13	0.21	0.05	0.01	0.62*	-0.17
spring	-0.15	-0.02	0.13	0.62	0.19	0.45*	-0.35
summer	-0.16	0.20	0.27	-0.06	0.51	0.70*	0.02
autumn	-0.30	0.21	0.33	0.95*	-0.02	0.96*	0.41*
winter	-0.22	0.20	0.58*	-0.08	0.35	0.66*	-0.46*
	RSS_{nv} below chemocline						
	T	S	DOC	POC	SAS	RSS_{tot}	SSI
1996 - 2021	0.09	0.19	0.39*	0.01	0.004	0.61*	-0.06
1996 - 2007	0.39*	-0.01	0.72*	n.m.	-0.05	0.81*	-0.11
2008 - 2013	-0.05	0.16	0.12	0.19	-0.03	0.46*	0.11
2014 - 2020	0.17	-0.02	0.27	-0.13	-0.16	0.65*	-0.20
spring	-0.29	0.08	0.24	0.05	n.m.	0.14	-0.03
summer	0.12	0.32	0.50*	-0.14	n.m.	0.72*	0.03
autumn	0.03	0.33	0.19	-0.11	0.002	0.65*	-0.25
winter	0.04	0.25	0.43	-0.02	n.m.	0.62*	0.43

2007; 2008-2013; 2014-2020). The Pearson correlation coefficients (r) for the strength of these relationships are part of Table 2, indicating a crucial role of water column stability as well as OM concentration impact on RSS including S_x^{2-} concentration and distribution.

DISCUSSION

The temporal and spatial dynamics of RSS in the RL water column, including total RSS (RSS_{tot}), non-volatile RSS (RSS_{nv}), and corresponding sulfur species like S_x^{2-} (measured as polysulfidic S^0 equivalents), show variability depending on the depth and physicochemical conditions. The results align with previous research on the diversity, abundance, and phylogeny of sulfate-reducing bacteria (SRB), which are the primary producers of RSS under anoxic conditions (Jørgensen *et al*., 1982), specifically in the water layer of RL below the chemocline (Čanković *et al*., 2017). This research suggested that the RL is a relatively dynamic area, exhibiting seasonal variations in the diversity and abundance of SRB communities both in the water column and in the sediment. Sulfate reduction normally occurs in fully anoxic sediments by SRB (Holmer and Storkholm, 2001). However, a water column with euxinic conditions and a high availability of organic carbon is also suitable for the growth of an important community of planktonic SRB (Jørgensen *et al*., 1979; Llorens-Marès *et al.*, 2015). The discussion in this study integrates observations from various water column conditions in the lake: stratified, weakly stratified, and holomictic conditions, and explores the influence of physicochemical parameters on RSS dynamics.

Physicochemical parameters and RSS dynamics

The pronounced thermocline at 9 meters and the halocline below 10 meters indicate a stable stratification of the water column in May 2019. The T profile shows a distinct subsurface maximum (Legović *et al.*, 1991; Izhitskiy *et al.*, 2021; Marguš *et al.*, 2023), which probably affects microbial activity and further sulfur speciation. The hypoxic conditions at 8 meters and anoxic conditions below 9 meters create an environment conducive to the accumulation of RSS_{tot} and S_x^{2-} (Čanković *et al.*, 2020; Simonović *et al.*, 2023). The increase in RSS_{tot} (up to 700 μM) and the presence of S_x^{2-} (up to 60 μM) in the anoxic layer are consistent with lower oxidation of sulfur compounds due to the lack of oxygen (Kamyshny *et al.*, 2011). Increased POC in the chemocline and deeper layers reduces light penetration (Čanković *et al.*, 2020), affecting the growth of PSB. PSB usually dominate in water column layers with stronger stratification at the sulfide-oxygen interface, higher amount of light, and lower presence of hydrogen sulfide as already found in RL (Čanković *et al.*, 2017) and similar stratified water columns of meromictic lakes such as Mahoney lake (British Columbia)

(Overmann *et al.*, 1996), Lake Cadagno (Switzerland) (Tonolla *et al.*, 2004; Danza *et al.*, 2018), and Lake Shunet (Siberia, Khakassia) (Rogozin *et al.*, 2012), as well as karstic Lake Cisó and Lake Vilar (Spain) (van Gemerden *et al.,* 1985). The opposite is found for GSB which prefer darker environments, higher sulfide concentration (van Gemerden *et al.,* 1985; Guerrero *et al.*, 1985), and often thrive beneath the PSB layer (Musat *et al.*, 2008) similar as reported for Lake Vilar (Spain) (Guerrero *et al*., 1985; Llorens-Marès *et al.*, 2015).

Ecological niche partitioning between GSB and PSB is considered to be mainly based on sulfide and oxygen concentrations, as well light availability (Abella *et al.*, 1980; Stomp *et al.*, 2007). Members of the genus *Chlorobium* (GSB) are obligate anaerobes with a limited metabolic capability, compared to many PSB that can tolerate, and in some cases even utilize oxygen, may also seek some distance from the oxic–anoxic interface, particularly if the interface is unstable (Canfield *et al.*, 2005). The dominance of PSB, which are motile and store sulfur intracellularly (Madigan *et al.*, 2017; Frigaard and Dahl, 2008) is usually dominated in the RL chemocline layer (Čanković *et al.* 2017; Čanković, 2018) similar as found for the spring/summer of 2019 when PSB abundance was more than 80 % of the total microbial population (Čanković, personal communication). PSB presence can be here indirectly proved by higher S_x^{2-} concentrations (Mateša *et al.*, 2024).

A weaker stratification in July 2020 is indicated by the absence of a distinct halocline and smaller differences in σ_T, as already described for RL (Ciglenečki *et al.*, 2015; Dominović *et al.,* 2023). This weaker stratification, combined with a thermocline at 9-10 meters, suggests a more mixed water column compared to spring 2019. Hypoxic conditions are present at 7 meters, with anoxic conditions from 8 to 12 meters. However, the overall lower concentrations of S_x^{2-} (1-6 µM) in summer 2020 suggest greater mixing between oxic and anoxic layers compared to spring 2019, because this process decreases the S_x^{2-} accumulation. Higher POC values above and/or at the chemocline indicate increased turbidity, which affects light availability and microbial activity (Čanković *et al.*, 2020), consequently influencing RSS_{nv}, i.e. S_x^{2-} concentrations. The presence of GSB is more prominent in this period (~ 33 % in the total microbial population, Čanković, personal communication), as GSB can tolerate sulfide better than PSB (Frigaard and Dahl, 2008) which then in the chemocline layer and below it was found in higher concentrations.

The complete mixing of the water column in October 2020, i.e. holomictic conditions results in vertically uniform T, S, and σ_T (Marguš *et al.*, 2015; Ciglenečki *et al.*, 2017). The lack of stratification leads to the equalization of physicochemical parameters throughout the water column. High oxygen levels at the surface are a result of algal blooms, particularly the intense bloom of the green alga *Picochlorum sp.* during that period (Simonović *et al.*, 2023), while the rest of the water column remains anoxic. The oxidation and precipitation of sulfide into colloidal and nanoparticulate S^0 (Bura-Nakić *et al.*, 2009; Marguš *et al.*, 2015, 2016) lowers the RSS_{tot} concentration to a maximum of 85 µM. Then, no S_x^{2-} is detected due to the complete mixing of the water column, leading to their decomposition and oxidation together with sulfide oxidation.

The highest light intensity (500 - 3300 lux), in the chemocline region during the summer of 2019, correlates with higher S_x^{2-} concentrations (especially measured in the layer below the chemocline) and the dominance of PSB, which, as already noted, requires more light and tolerate lower sulfide concentrations for their activity (Frigaard and Dahl, 2008; Madigan *et al.*, 2017). Conversely, during the winter and summer of 2020, when light intensity was low at the chemocline and SSI relatively high (especially in January 2020), the S_x^{2-} concentrations were also lower. The main factors determining the growth of PSB in similar stratified, sulfur rich lake environments, such as Mahoney lake (British Columbia) and lake Ciso in Spain, are light and HS^- (Overmann *et al.*, 1991). PSB and GSB play different roles based on light availability in RL. PSB, which are active in well-lit stratified layers, appear to contribute to higher S_x^{2-} concentrations. In contrast, according to our study, GSB, which are more active under euxinic conditions without light, correlate with lower S_x^{2-} levels.

The stability of the water column, as indicated by the SSI, impacts the distribution of S_x^{2-}. High SSI values correlate with stable conditions and higher S_x^{2-} concentrations which may be explained by decreased mixing and oxidation. In contrast, low SSI values and periods of mixing lead to decreased S_x^{2-} levels as a result of increased exchange between oxic and anoxic layers and subsequent oxidation of RSS. This is in agreement with findings showing that more stable stratification contributes to a higher abundance of PSB layers in the stratified water column of Lake Cadagno in Switzerland (Tonolla *et al.*, 2004, 2005) as well as in RL (Pjevac *et al*., 2015; Čanković *et al.*, 2017, 2019, 2020). Persistent and stable stratification in Mahoney Lake keeps PSB biomass lost due to sedimentation and decay at very low levels (Overmann *et al.*, 1991). Changing environmental conditions within the chemocline in lake Cadagno lead to pronounced shifts both in dominance (from PSB to GSB) and the abundance between GSB populations (Tonolla *et al.*, 2005). Similar community shifts were already found in the RL water column after the holomixing period in 2011 (Pjevac *et al.*, 2015; Čanković *et al*., 2020).

Long-term data of the RSS_{nv} in chemocline and below the chemocline layer

Long-term monitoring of RSS concentrations in the RL water column, spanning from 1996 to 2021, has provided valuable insights into the dynamics of RSS_{nv} in relation to the physical and chemical parameters studied in this paper. This unique data reveals complex patterns of periodic changes in RSS_{nv} concentrations, which correlate with variations in DOC, POC and other relevant factors.

The long-term data show that RSS_{nv} concentrations in the chemocline and below the chemocline exhibit periodic changes that correlate with water column stability and OM dynamics. Higher RSS concentrations are typically observed during periods of pronounced water column stability, when stratification is stronger and euxinic conditions prevail, as already shown for RL (Bura-Nakić *et al.*, 2009; Ciglenečki *et al.*, 2017; Čanković *et al.*, 2019). Under such conditions, the volatile fraction of RSS, i.e. sulfide, predominates in the euxinic layer (Bura-Nakić *et al.*, 2009; Ciglenečki *et al.*, 2005). However, according to Fig. 4, RSS_{nv} concentrations are the highest at times when the stability of the water column is the greatest. This is consistent with the assumption that most of the RSS_{nv} is generated by the activity of PSB, which prefers highly stratified conditions, as discussed above.

At the chemocline layer RSS_{nv} concentrations show weak correlations with DOC (r = 0.29) and SAS (r = 0.29) but a moderate correlation with RSS_{tot} (r = 0.48). This suggests that while there is some association between OM and RSS, it is not strongly dominant in this layer. It appears that in this layer a crucial fraction of RSS_{tot} is RSS_{nv} i.e. elemental S^0 including S^0 from polysulfides.

Similar findings are valid for spring when also a moderate correlation between RSS_{nv} and RSS_{tot} was found (r = 0.45). However, in summer, stronger correlations with RSS_{tot} (r = 0.70) and good correlations with DOC (r = 0.58) suggest that biological activity and OM release are important contributors to RSS_{nv}. In fall, the correlation with RSS_{tot} is excellent (r = 0.96), indicating that RSS_{nv} (attributable to the presence of S^0) is largely comprised of the non-volatile fraction of RSS_{tot} during this time when vertical mixing occurs and oxidation of sulfide ended with precipitation of colloidal and nanoparticulate S^0 (Bura-Nakić *et al.*, 2009; Marguš *et al.*, 2015; Čanković *et al.*, 2020). In winter, a good correlation with RSS_{tot} (r = 0.66) and DOC (r = 0.58) probably points to the presence of S^0 including polysulfidic S^0 formed by chemoautotrophic activities as indicated in study of Kamyshny *et al.* (2011). The presence of colloidal S^0 was indicated by a slightly milky appearance of the water from the chemocline, while large amounts of elemental S^0 are, however, present as polysulfides in the layer below, similar as reported for Solar Lake, Sinai (Jørgensen *et al.*, 1979). Our preliminary measurements also confirmed dynamic transformations of RSS during the day, when the maximal concentration of polysulfidic S^0 was measured in the afternoon just after maximal light intensities reached the chemocline (Table 1, June 28, 2020), indicating shifts toward more oxidation processes similar as found in Lake Kinneret (Israel) (Avetisyan *et al.,* 2019), which needs further investigation.

In the layer below the chemocline, RSS_{nv} shows a stronger correlation with RSS_{tot} (r = 0.61), indicating a more direct relationship with the total sulfur pool, while the correlation with DOC (r = 0.39) is weaker but still pronounced. It should be noted that there is a connection between anoxygenic photosynthesis and sulfate reduction in the water column. This connection confirmed an unexpected and direct link between the anoxygenic phototrophic population and the microbial food web in the upper mixolimnion of Mahoney Lake (Canfield *et al.*, 2005).

In summer, strong correlations with RSS_{tot} (r = 0.66) and DOC (r = 0.58) also suggest crucial biological contributions to RSS_{nv}. Autumn correlations with RSS_{tot} (r = 0.65) are good, but the correlation with POC (r = 0.95) highlights the role of particulate matter in RSS_{nv} dynamics, which is consistent with water column conditions during mixing process when sediments enriched with OM (Ciglenečki *et al.*, 2006) can be suspended through the water column to the surface of the lake (Marguš *et al.*, 2015). In winter, there is a good correlation with RSS_{tot} (r = 0.62), indicating that RSS_{nv} is mainly composed of sulfur species that are not easily volatilized.

Generally, our study highlighted that the stability of the water column plays a crucial role in determining RSS concentrations. High stability periods, where stratification is strong, generally correlate with higher RSS_{nv} concentrations which, in the case of RL, are an important fraction of RSS_{tot} (Bura-Nakić *et al.*, 2009). This is likely due to the favorable conditions for PSB, which thrive in stable, stratified environments and contribute to the production of non-volatile sulfur species (Mateša *et al.*, 2024, and references therein).

In spring and winter, RSS_{nv} concentrations tend to be lower and more variable, reflecting reduced biological activity and interaction with OM. In summer, higher RSS_{nv} concentrations correlate with increased light intensity and biological activity, especially by PSB, which appear to notable contribute to the formation of polysulfides in the RL water column (Mateša *et al.*, 2024). The strong correlation with POC and RSS_{tot} in autumn suggests that mixing events and resuspension of OM from sediments play a crucial role in RSS_{nv} speciation.

On the other hand, the results of this work support our previous conclusions (Bura-Nakić *et al.*, 2009), which hypothesized the anoxic hypolimnion of the RL as a bioreactor for elemental sulfur, where interactions between polysulfidic S^0 and OM are critical for the stability and dynamics of RSS in the water column, with a strong impact on sediment geochemistry (Mihelčić *et al.*, 1996; Ciglenečki *et al.*, 2006) as well. Overall, our results provide a framework for understanding the interplay among the physicochemical characteristics of the stratified euxinic environment, RSS speciation, and microbial ecology, serving as a foundation for more detailed future research.

CONCLUSIONS

The dynamics of sulfur species in the RL water column, particularly the concentrations of non-volatile

RSS (RSS_{nv}) attributable to elemental S, including polysulfidic S^0 ($S^0_{polysulfidic}$), reveal seasonal and spatial variability, which is influenced by physicochemical parameters and microbial activity. The highest concentrations of polysulfidic S^0 are observed under conditions of pronounced stable stratification, where the water column is clearly divided into oxic and anoxic layers, with a well-defined chemocline. Under these conditions, polysulfidic S^0 reaches its peak in the layer just below the chemocline. This is attributed to the high light intensity and the dominance of PSB. PSB, which are active at the oxic-anoxic boundary, contribute to the biologically mediated production of S^0, and thus influence the formation of S_x^{2-}.

Analysis of long-term data (1996-2021) on RSS_{nv} concentrations reveals cyclical patterns related to water column stability and varying physical and chemical conditions. RSS_{nv}, which is assumed to originate largely from the degradation or hydrolysis of S_x^{2-}, shows fluctuations that correlate with the stability of the RL water column indicated by the SSI. Periods of high RSS_{nv} concentrations often coincide with more stable conditions, while lower concentrations are associated with less stable or mixed conditions.

RSS_{nv} concentrations show a remarkable association with OM over different seasons and time periods. This suggests a possible interaction between RSS and OM, either through direct chemical interactions or through a common origin, where both may be influenced by similar environmental factors. The presence of OM may influence the distribution and speciation of RSS, potentially affecting the entire sulfur cycle in the water column.

Overall, the study highlights the complex interrelationship between physicochemical parameters, microbial activity and sulfur species in the RL water column, emphasizing how this dynamic can be extrapolated to other similar stratified ecosystems. In such systems in general, the biogeochemical cycling of sulfur and carbon varies based on water column chemistry and the proximity of prominent redox interfaces to light, which directly impacts microbial activity. Understanding these dynamics is crucial to elucidate the role of RSS in aquatic environment in general and their interaction with OM, and serve as a foundation for modeling the biogeochemical sulfur cycle within the stratified marine water column. Further exploration of these relationships will improve our understanding of the sulfur cycle and its impact on the environment, particularly in relation to the spread of deoxygenation in coastal waters due to climatic variability.

ACKNOWLEDGEMENTS

We would like to thank the Croatian Science Foundation for supporting this work through the project IP-2018-01-1717-MARRES and partly through the project IP-2024-05-2377-ISO-ZOKO. The authors thank all those who helped with the sampling of the RL water column, as well as SAS measurements, and J. Dautović and Z. Zovko for DOC and POC analyses. Finally, we thank the reviewers and the editors for their constructive comments that notably enhanced the manuscript.

AUTHOR CONTRIBUTIONS

Sarah Mateša - Conceptualization; Data curation; Formal analysis; Methodology; Visualization; Writing - Review & Editing (original draft and final version). Iva Dominović - Data curation; Formal analysis; Review-Editing (original draft). Marija Marguš - Data curation; Review-Editing (original draft). Irena Ciglenečki - Conceptualization; Methodology; Supervision; Validation; Writing - Review & Editing (original draft and final version); Funding acquisition; Project administration; Resources.

REFERENCES

Abella, C., Montesinos, E., Guerrero, R. 1980. Field studies on the competition between purple and green sulfur bacteria for available light (Lake Siso, Spain). *In* Shallow lakes contributions to their limnology (eds. H. Dokulil, H. Metz, D. Jewson). Developments in Hydrobiology, vol 3. Springer, Dordrecht. pp. 173-181. https://doi.org/10.1007/978-94-009-9206-1_26

Avetisyan, K., Eckert, W., Findlay, A.J., Kamyshny, A. 2019. Diurnal variations in sulfur transformations at the chemocline of a stratified freshwater lake. Biogeochemistry, 146, 83-100. https://doi.org/10.1007/s10533-019-00601-5

Barbash, J.E., Reinhardt, M. 1989. Reactivity of sulfur nucleophiles towards halogenated organic compounds in natural waters. *In* Biogenic sulphur in the environment (eds. E.S. Saltzman, W.J. Cooper). American Chemical Society. pp. 101.

Boulegue, J. 1978. Solubility of elemental sulfur in water at 298 K. Phosphorus Sulfur and Silicon and the Related Elements, 5(1), 127-128. https://doi.org/10.1080/03086647808069875

Bura-Nakić, E., Helz, G.R., Ciglenečki, I., Ćosović, B. 2009. Reduced sulfur species in a stratified seawater lake (Rogoznica Lake, Croatia); seasonal variations and argument for organic carriers of reactive sulfur. Geochimica et Cosmochimica Acta, 73(13), 3738-3751. https://doi.org/10.1016/j.gca.2009.03.037

Canfield, D.E., Kristensen, E., Thamdrup, B. 2005. Microbial ecosystems. Advances in Marine Biology, 48, 465-506. https://doi:10.1016/s0065-2881(05)48013-x

Ciglenečki, I., Kodba, Z., Ćosović, B. 1996. Sulfur species in Rogoznica Lake. Marine Chemistry, 53(1-2), 101-110. https://doi.org/10.1016/0304-4203(96)00016-3

Ciglenečki, I., Ćosović, B. 1997. Electrochemical determination of thiosulfate in seawater in the presence of elemental sulfur and sulfide. Electroanalysis, 9(10), 775-780. https://doi.org/10.1002/elan.1140091009

Ciglenečki, I., Kodba, Z., Viličić, D., Ćosović, B. 1998. Sesonal variation of anoxic conditions in the Rogoznica Lake. Croatica Chemica Acta, 71(2), 217-232.

Ciglenečki, I., Carić, M., Kršinić, F., Viličić, D., Ćosović, B. 2005. The extinction by sulfide-turnover and recovery of a naturally eutrophic, meromictic seawater lake. Journal of Marine Systems, 56(1-2), 29-44. https://doi.org/10.1016/j.jmarsys.2004.08.006

Ciglenečki, I., Pichler, S., Prohić, E., Ćosović, B. 2006. Distribution of redox-sensitive elements in bottom waters, porewaters and sediments of Rogoznica Lake (Croatia) in both oxic and anoxic conditions. Water, Air, and Soil Pollution: Focus, 6, 537-545. https://doi.org/10.1007/s11267-006-9037-2

Ciglenečki, I., Marguš, M., Bura-Nakić, E., Milanović, I. 2014. Electroanalytical methods in characterization of sulfur species in aqueous environment. Journal of Electrochemical Science and Engineering, 4(4), 155-163. https://doi.org/10.5599/jese.2014.0053

Ciglenečki, I., Janeković, I., Marguš, M., Bura-Nakić, E., Carić, M., Ljubešić, Z., Batistić, M. *et al.* 2015. Impacts of extreme weather events on highly eutrophic marine ecosystem (Rogoznica Lake, Adriatic coast). Continental Shelf Research, 108, 144-155. https://doi.org/10.1016/j.csr.2015.05.007

Ciglenečki, I., Ljubešić, Z., Janeković, I., Batistić, M. 2017. Rogoznica Lake, a euxinic marine lake on the Adriatic Coast (Croatia) that fluctuates between anoxic holomictic and meromictic conditions. *In* Ecology of Meromictic Lakes (eds. R.D. Gulati, E.S. Zadereev, A.G. Degermendzhi). Springer. pp. 125-154.

Ciglenečki, I., Orlović-Leko, P., Vidović, K., Simonović, N., Marguš, M., Dautović, J., Mateša, S. *et al.* 2023. The possibilities of voltammetry in the study reactivity of dissolved organic carbon (DOC) in natural waters. Journal of Solid State Electrochemistry, 27, 1781-1793. https://doi.org/10.1007/s10008-023-05423-y

Čanković, M. 2018. Molecular characterisation of the prokaryotic communities in the euxinic environment of Rogoznica Lake (in Croatian). PhD thesis, University of Zagreb, 111 pp.

Čanković, M., Sviličić Petrić, I., Marguš, M., Ciglenečki, I. 2017. Spatio-temporal dynamics of sulfate-reducing bacteria in extreme environment of Rogoznica Lake revealed by 16S rRNA analysis. Journal of Marine Systems, 172, 14-23. https://doi.org/10.1016/j.jmarsys.2017.03.003

Čanković, M., Žučko, J., Dupčić Radić, I., Janeković, I., Sviličić Petrić, I., Ciglenečki, I., Collins, G. 2019. Microbial diversity and long-term geochemical trends in the euxinic zone of a marine, meromictic lake. Systematic and Applied Microbiology, 42(6), 126016, 13. https://doi.org/10.1016/j.syapm.2019.126016

Čanković, M., Žučko, J., Petrić, I., Marguš, M., Ciglenečki, I. 2020. Impact of euxinic holomictic conditions on prokaryotic assemblages in a marine meromictic lake. Aquatic Microbial Ecology, 84, 141-154. https://doi.org/10.3354/ame01931

Ćosović, B., Vojvodić, V. 1982. The application of ac polarography to the determination of surface-active substances in seawater. Limnology and Oceanography, 27(2), 361-369. https://doi.org/10.4319/lo.1982.27.2.0361

Ćosović, B., Vojvodić, V. 1998. Voltammetric analysis of surface active substances in natural seawater. Electroanalysis, 10(6), 429-434. https://doi.org/10.1002/(SICI)1521-4109(199805)10:6<429::AID-ELAN429>3.0.CO;2-7

Danza, F., Ravasi, D., Storelli, N., Roman, S., Ludin, S., Bueche, M., Tonolla, M. 2018. Bacterial diversity in the water column of meromictic Lake Cadagno and evidence for seasonal dynamics. PLoS ONE, 13(12), e0209743. https://doi.org/10.1371/journal.pone.0209743

Dautović, J., Strmečki, S., Pestorić, B., Vojvodić, V., Plavšić, M., Krivokapić, S. 2012. Organic matter in the karstic enclosed bay (Boka Kotorska Bay, South Adriatic Sea). Influence of freshwater input. Fresenius Environmental Bulletin, 21(4), 995-1006.

Dautović, J., Vojvodić, V., Tepić, N., Ćosović, B., Ciglenečki, I. 2017. Dissolved organic carbon as potential indicator of global change: a long-term investigation in the northern Adriatic. Science of the Total Environment, 587-588, 185-195. https://doi.org/10.1016/j.scitotenv.2017.02.111

Dominović, I., Dutour-Sikirić, M., Marguš, M., Bakran-Petricioli, T., Petricioli, D., Geček, S., Ciglenečki, I. 2023. Deoxygenation and stratification dynamics in a coastal marine lake. Estuarine, Coastal and Shelf Science, 291, 108420. https://doi.org/10.1016/j.ecss.2023.108420

Dominović, I., Marguš, M., Bakran-Petricioli, T., Petricioli, D., Ciglenečki, I., Vilibić, I. 2024. Observing lake-sea tidally driven water exchange in Lake Zmajevo Oko (Rogoznica, Croatia). Acta Adriatica, 65(2), 179-192. https://doi.org/10.32582/aa.65.2.6

Findlay, A.J. 2016. Microbial impact on polysulfide dynamics in the environment. FEMS Microbiology Letters, 363 (11), 1-12. https://doi.org/10.1093/femsle/fnw103

Frigaard, N.U., Dahl, C. 2008. Sulfur metabolism in phototrophic sulfur bacteria. *In* Advances in Microbial Physiology (ed. R.K. Poole). Elsevier. pp. 104-200.

Giggenbach, W. 1972. Optical spectra and equilibrium distribution of polysulfide ions in aqueous solution at 20. deg. Inorganic Chemistry, 11(6), 1201-1207. https://doi.org/10.1021/ic50112a009

Guerrero, R., Montesinos, E., Pedrós-Alió, C., Esteve, I., Mas, J., van Gemerden H., Hofman P.A.G. *et al.* 1985. Phototrophic sulfur bacteria in two Spanish lakes: vertical distribution and limiting factors. Limnology and Oceanography, 30(5), 919-931. https://doi.org/10.4319/lo.1985.30.5.0919

Helz, G.R., Bura-Nakić, E., Mikac, N., Ciglenečki, I. 2011. New model for molybdenum behavior in euxinic waters. Chemical Geology, 284(3-4), 323-332. https://doi.org/10.1016/j.chemgeo.2011.03.012

Helz, G.R. 2014. Activity of zero-valent sulfur in sulfidic natural waters. Geochemical Transactions, 15, 13. https://doi.org/10.1186/s12932-014-0013-x

Helz, G.R., Erickson, B.E., Vorlicek, T.P. 2014. Stabilities of thiomolybdate complexes of iron; implications for retention of essential trace elements (Fe, Cu, Mo) in sulfidic waters. Metallomics, 6(6), 1131-1140. https://doi.org/10.1039/C3MT00217A

Holmer, M., Storkholm, P. 2001. Sulphate reduction and sulphur cycling in lake sediments: a review. Freshwater Biology, 46(4), 431-451. https://doi.org/10.1046/j.1365-2427.2001.00687.x

Idso, S.B. 1973. On the concept of lake stability. Limnology and Oceanography, 18(4), 681-683. https://doi.org/10.4319/lo.1973.18.4.0681

Izhitskiy, A.S., Kirillin, G.B., Goncharenko, I.V., Kurbaniyazov, A.K., Zavialov, P.O. 2021. The world's largest heliothermal lake newly formed in the Aral Sea basin. Environmental Research Letters, 16, 115009. https://doi.org.10.1088/1748-9326/ac2d66

Jørgensen, B.B., Kuenen, J.G., Cohen, Y. 1979. Microbial transformations of sulfur compounds in a stratified lake (Solar lake, Sinai). Limnology and Oceanography, 24(5), 799-822. https://doi.org/10.4319/lo.1979.24.5.0799

Jørgensen, B.B. 1982. Mineralization of organic matter in the sea bed- the role of sulphate reduction. Nature, 296, 643-645. https://doi: 10.1038/296643a0

Kamyshny, A., Goifman, A., Gun, J., Rizkov, D., Lev, O. 2004. Equilibrium distribution of polysulfide ions in aqueous solutions at 25 degrees C: a new approach for the study of polysulfides equilibria. Environmental Science & Technology, 38(24), 6633-6644. https://doi.org/10.1021/es049514e

Kamyshny, A., Zerkle, A.L., Mansaray, Z.F., Ciglenečki, I., Bura-Nakić, E., Farquhar, J., Ferdelman, T.G. 2011. Biogeochemical sulfur cycling in the water column of a shallow stratified sea-water lake: speciation and quadruple sulfur isotope composition. Marine Chemistry, 127(1-4), 144-154. https://doi.org/10.1016/j.marchem.2011.09.001

Kariuki, S., Morra, M.J., Umiker, K.J., Cheng, I.F. 2001. Determination of total ionic polysulfides by differential pulse polarography. Analytical Chimica Acta, 442(2), 277-285. https://doi.org/10.1016/S0003-2670(01)01171-0

Kleinjan, W.E., de Keizer, A., Janssen, A.J.H. 2003. Biologically produced sulfur. *In* Elemental sulfur and sulfur-rich compounds I (eds. R. Steudel). Springer. pp. 167-188.

Kleinjan, W.E., de Keizer, A., Janssen, A.J.H. 2005. Kinetics of the chemical oxidation of polysulfide anions in aqueous solution. Water Research, 39(17), 4093-4100. https://doi.org/10.1016/j.watres.2005.08.006

Kršinić, F., Carić, M., Viličić, D., Ciglenečki, I. 2000. The calanoid copepod *Acartia italica* Steuer, phenomenon in the small saline Lake Rogoznica (Eastern Adriatic coast). Journal of Plankton Research, 22(8), 1441-1464. https://doi.org/10.1093/plankt/22.8.1441

Kushkevych, I., Procházka, J., Gajdács, M., Rittmann, S.K.-M.R., Vítězová, M. 2021. Molecular physiology of anaerobic phototrophic purple and green sulfur bacteria. International Journal of Molecular Sciences, 22(12), 6398. https://doi.org/10.3390/ijms22126398

Legović, T., Gržetić, Z., Žutić, V. 1991. Subsurface temperature maximum in a stratified estuary. Marine Chemistry, 32(2-4), 163-170. https://doi.org/10.1016/0304-4203(91)90035-U

Llorens-Marès, T., Yooseph, S., Goll, J., Hoffman, J., Vila-Costa, M., Borrego, C.M., Dupont, C.L. *et al*. 2015. Connecting biodiversity and potential functional role in modern euxinic environments by microbial metagenomics. The ISME Journal, 9(7), 1648-1661. https://doi.org/10.1038/ismej.2014.254

Madigan, M.T., Martinko, J.M., Parker, J. 2017. Brock biology of microorganisms. Pearson, 1064 pp.

Marguš, M., Morales-Reyes, I., Bura-Nakić, E., Batina, N., Ciglenečki, I. 2015. The anoxic stress conditions explored at the nanoscale by atomic force microscopy in highly eutrophic and sulfidic marine lake. Continental Shelf Research, 109, 24-34. https://doi.org/10.1016/j.csr.2015.09.001

Marguš, M., Coha, I., Ciglenečki, I. 2016. Voltammetric, dynamic light scattering (DLS) and electrophoretic mobility characterization of FeS nanoparticles (NPs) in different electrolyte solutions. Journal of Solid State Electrochemistry, 20(11), 2981-2989. https://doi.org/10.1007/s10008-016-3354-9

Marguš, M., Ahel, M., Čanković, M., Ljubešić, Z., Terzić, S., Hodak Kobasić, V., Ciglenečki, I. 2023. Phytoplankton pigment dynamics in marine lake fluctuating between stratified and holomictic euxinic conditions. Marine Pollution Bulletin, 191, 114931. https://doi.org/10.1016/j.marpolbul.2023.114931

Mateša, S., Marguš, M., Šegota, S., Ciglenečki, I. 2024. Characterization of polysulfides (S_x^{2-}) in seawater euxinic conditions by electroanalytical methods. Marine Chemistry, 262, 104398. https://doi.org/10.1016/j.marchem.2024.104398

Meyer, K.M., Kump, L.R. 2008. Oceanic euxinia in Earth history: causes and consequences. Annual Review of Earth and Planetary Sciences, 36, 251-288. https://doi.org/10.1146/annurev.earth.36.031207.124256

Mifka, B., Telišman Prtenjak, M., Kuzmić, J., Čanković, M., Mateša, S., Ciglenečki, I. 2022. Climatology of dust deposition in the Adriatic Sea; a possible impact on marine production. Journal of Geophysical Research: Atmospheres, 127(7), e2021JD035783. https://doi.org/10.1029/2021JD035783

Mihelčić, G., Šurija, B., Juračić, M., Barišić, D., Branica, M. 1996. History of the accumulation of trace metals in sediments from the saline Rogoznica Lake (Croatia). Science of the Total Environment, 182(1-3), 105-115. https://doi.org/10.1016/0048-9697(95)05058-2

Musat, N., Halm, H., Winterholler, B., Hoppe, P., Peduzzi, S., Hillion, F., Horreard, F. *et al.* 2008. A single-cell view on the ecophysiology of anaerobic phototrophic bacteria. Proceedings of the National Academy of Sciences of the United States of America, 105(46), 17861-17866. https://doi.org/10.1073/pnas.0809329105

Overmann, J., Beatty, J.T., Hall, K.J., Pfennig, N., Northcote, T.G. 1991. Characterization of a dense, purple sulfur bacterial layer in a meromictic salt lake. Limnology and Oceanography, 36(5), 846-859. https://doi.org/10.4319/lo.1991.36.5.0846

Overmann, J., Beatty, J.T., Krause, H.R., Hall, K.J. 1996. The sulfur cycle in the chemocline of a meromictic salt lake. Limnology and Oceanography, 41(1), 147-156. https://doi.org/10.4319/lo.1996.41.1.0147

Panđa, L. 2020. Geospatial technologies in modelling and promoting of protected areas on the example of lake Zmajevo oko (in Croatian). Master's thesis, University of Zadar, 98 pp.

Pjevac, P., Korlević, M., Berg, J.S., Bura-Nakić, E., Ciglenečki, I., Amann, R., Orlić, S. 2015. Community shift from phototrophic to chemotrophic sulfide oxidation following anoxic holomixis in a stratified seawater Lake. Applied and Environmental Microbiology, 81(1), 298-308. https://doi.org/10.1128/AEM.02435-14

Rogozin, D.Y., Zykov, V.V., Degermendzhi, A.G. 2012. Ecology of purple sulfur bacteria in the highly stratified meromictic Lake Shunet (Siberia, Khakassia) in 2002-2009. Microbiology, 81, 727-735. https://doi.org/10.1134/S0026261712060148

Simonović, N., Dominović, I., Marguš, M., Matek, A., Ljubešić, Z., Ciglenečki, I. 2023. Dynamics of organic matter in the changing environment of a stratified marine lake over two decades. Science of The Total Environment, 865, 161076. https://doi.org/10.1016/j.scitotenv.2022.161076

Stomp, M., Huisman, J., Stal, L.J., Matthijs, H.C.P. 2007. Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. The ISME Journal, 1(4), 271-282. https://doi.org/10.1038/ismej.2007.59

Stumm, W., Morgan, J.J. 1996. Aquatic chemistry: chemical equilibria and rates in natural waters. Wiley Interscience, 1040 pp.

Tonolla, M., Peduzzi, S., Demarta, A., Peduzzi, R., Hahn, D. 2004. Phototropic sulfur and sulfate-reducing bacteria in the chemocline of meromictic Lake Cadagno, Switzerland. Journal of Limnology, 63(2), 161-170. https://doi.org/10.4081/jlimnol.2004.161

Tonolla, M., Peduzzi, R., Hahn, D. 2005. Long-term population dynamics of phototrophic sulfur bacteria in the chemocline of Lake Cadagno, Switzerland. Applied and Environmental Microbiology, 71(7), 3544-3550. https://doi.org/10.1128/AEM.71.7.3544-3550.2005

Udovičić, M., Baždarić, K., Bilić-Zulle, L., Petrovečki, M. 2007. What we need to know when calculating the coefficient of correlation? Biochemia Medica, 17(1), 10-15. https://doi.org/10.11613/BM.2007.002

van Gemerden, H., Montesinos, E., Mas, J., Guerrero, R. 1985. Diel cycle of metabolism of phototrophic purple sulfur bacteria in Lake Cisó (Spain). Limnology and Oceanography, 30(5), 932-943. https://doi.org/10.4319/lo.1985.30.5.0932

Zadereev, E.S., Boehrer, B., Gulati, R.D. 2017. Introduction: meromictic lakes, their terminology and geographic distribution. *In* Ecology of meromictic lakes (eds. R.D. Gulati, E.S. Zadereev, A.G. Degermendzhi). Springer. pp. 1-11. https://doi.org./10.1007/978-3-319-49143-1_1