Spectrophotometric determination of the distribution of phototrophic microorganisms in meromictic reservoirs of the White Sea in 2025

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Introduction

On the coast of the White Sea there are some reservoirs that are gradually becoming isolated from the sea. In such reservoirs, fresh water enters from the surface while seawater remains below. Due to the lack of mixing between water layers caused by differences in water density, distinct stratification occurs. An oxygen-rich upper layer adjoins an anaerobic lower zone enriched with hydrogen sulfide. The distribution of phototrophic microorganisms (such as phytoplankton, cyanobacteria, green and purple sulfur bacteria) varies depending on water properties and changes with increasing depth within the reservoir.

Herein we present the findings obtained through spectrophotometric analysis of microbial photosynthetic pigments conducted during summer field campaigns in 2025 in the meromictic lakes Elovoe and Trekhtzvetnoe located along the Kandalaksha Bay of the White Sea.

Materials and methods

Natural water samples with phototrophic microorganisms were collected in June 2025 from reservoirs located on the coast of Kandalaksha Bay of the White Sea. The Lake Trekhtzvetnoe (66°35'31.60"N, 32°58'45.39"E, area 5 ha, maximum depth 7.5 m) and the Lake Elovoe (66°28'54.60"N, 33°16'56.44"E, area 3.5 ha, maximum depth 5.5 m) are coastal meromictic lakes, cut off from the White sea due to the postglacial coastal uplift. Both lakes are characterized by stable hydrological stratification and consist of a bottom relict seawater preserved since the time when the reservoir was connected to the sea; a surface freshwater layer; and a gradient zone (chemocline) between them. Simultaneously with sampling, water temperature, salinity, pH and Eh, dissolved oxygen content and irradiance were measured *in situ* at different depths.

Extraction of pigments from Chl-containing cells

To extract pigments from chlorophyll (Chl)-containing algal and bacterial cells, the biomass of phototrophic organisms was concentrated by filtration through a GF/F filter with a pore diameter of 0.45 μm using a vacuum pump. The resulting filter with biomass was cut into small pieces and placed in 100% acetone. The volume of the filtered sample and the added acetone was recorded to calculate the chlorophyll a concentration. The tubes were incubated in the dark at 4°C for at least 2 hours.

Extraction of pigments from green sulphur bacteria with chlorosomal bacteriochlorophylls (BChl)

To obtain pigment extracts from the cells of anoxygenic phototrophic bacteria, 4 ml of a mixture of acetone and methanol (7:2) were added to 1 ml of water sample. The tubes were incubated in the dark at 4°C for at least 24 hours.

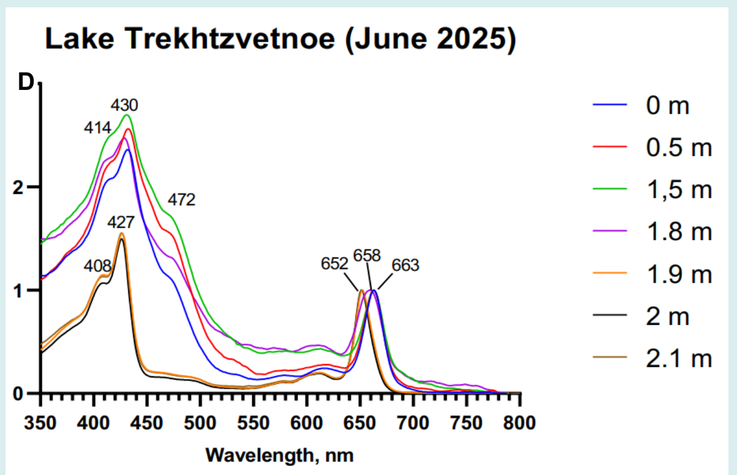
To remove solid particles, all extractions were centrifuged at 3000 rpm for 5 min in an Eppendorf 5804 centrifuge (Germany). Absorption spectra were recorded using a SOLAR PB2201 spectrophotometer (Belarus) in quartz cuvette with an optical path length of 1 cm. If the absorbance value exceeded 1, the extract was diluted.

Concentrations of photosynthetic pigments

To calculate the chlorophyll *a* concentration, the Jeffrey & Humphrey (1975) formula was used: C(Chl *a*) = 11.43 * D663 - 0.64 * D630, [μg/ml], where E663 and E630 are absorbances at 663 and 660 nm. The formula for calculation of the BChl concentration in an arbitrary solvent (with only one BChl type present in the solution) is C (BChl = A * D655 [μg/ml], where D is the absorbance at the band maximum (655 nm), and empirical coefficient A = 27,9 (mg cm)/L for BChl d and 42,7 (mg cm)/L for BChl e. For the mixture of BChl d and BChl e we estimated proportion of each BChl using blue bands in absorption spectra.

*Extinction coefficients of bacteriochlorophylls d and e in organic solvents for quantitative spectrophotometric determination of pigments of phototrophic green sulphur bacteria / A. A. Zhiltsova, E. D. Krasnova, D. A. Voronov et al. // Optics and Spectroscopy. Vol. 132, no. 3. P. 214–222.

1.Absorption spectra of pigments: oxygen-reach water



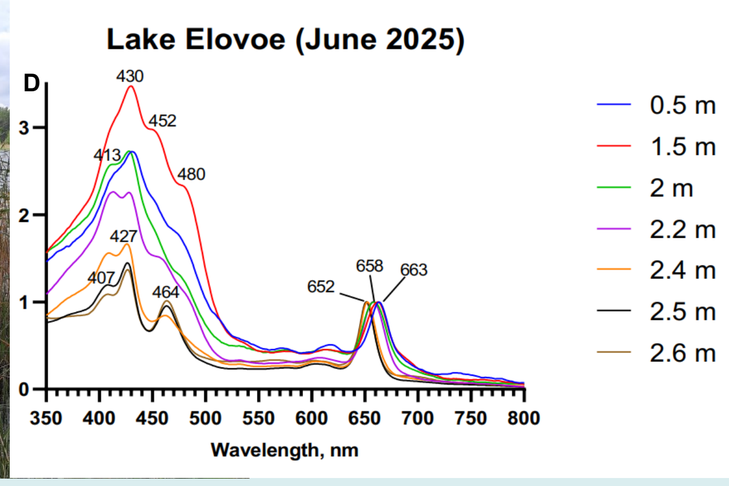


Figure 1. Absorption spectra of acetone extractions for different depths of the Lakes Trekhtzvetnoe and Elovoe from surface to chemocline.

2. Absorption spectra of pigments: anoxic zone

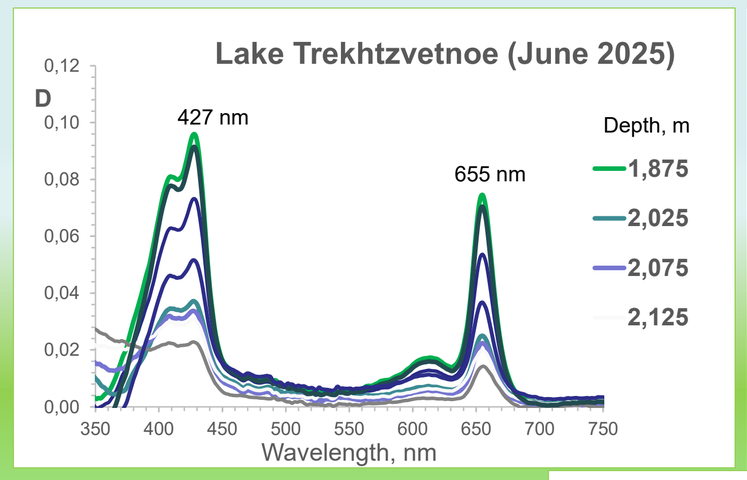
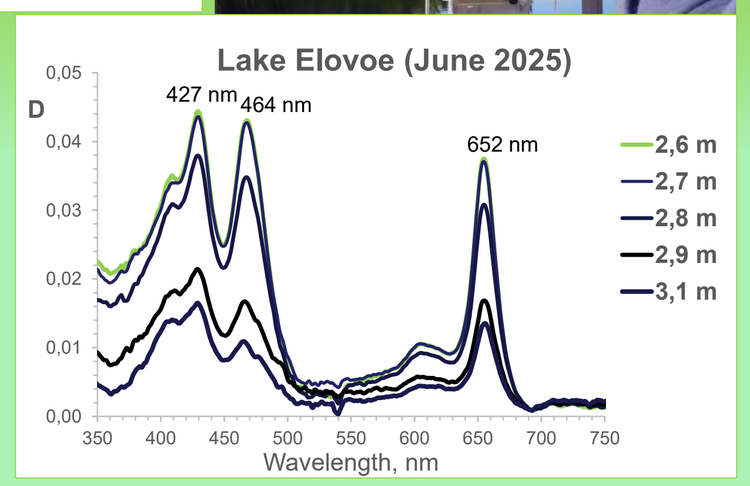
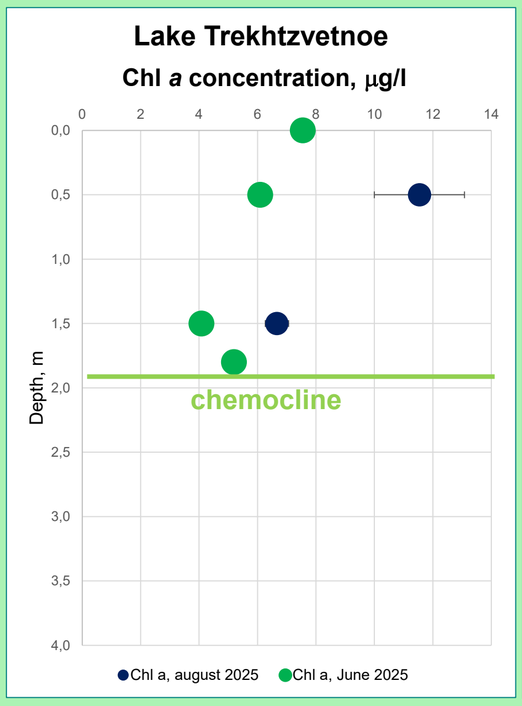
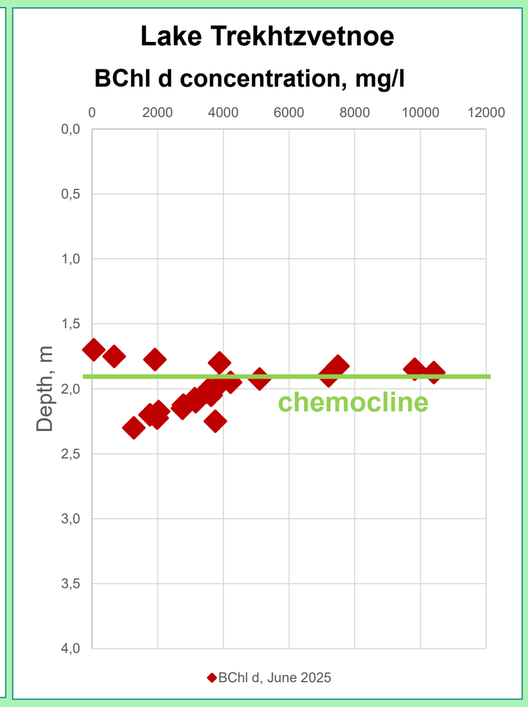


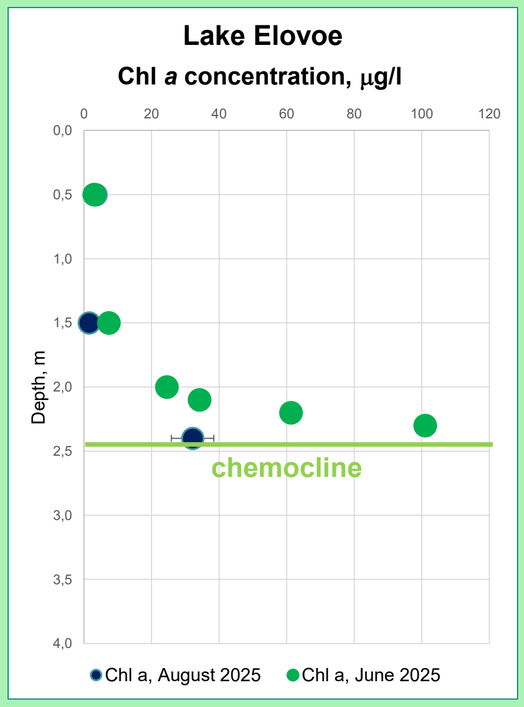
Figure 2. Absorption spectra of acetone-ethanol extractions for different depths of the Lakes Trekhtzvetnoe and Elovoe within chemocline zone.



3. Depth distribution of photosynthetic pigments: Chl *a* and BChl (*d*+*e*)







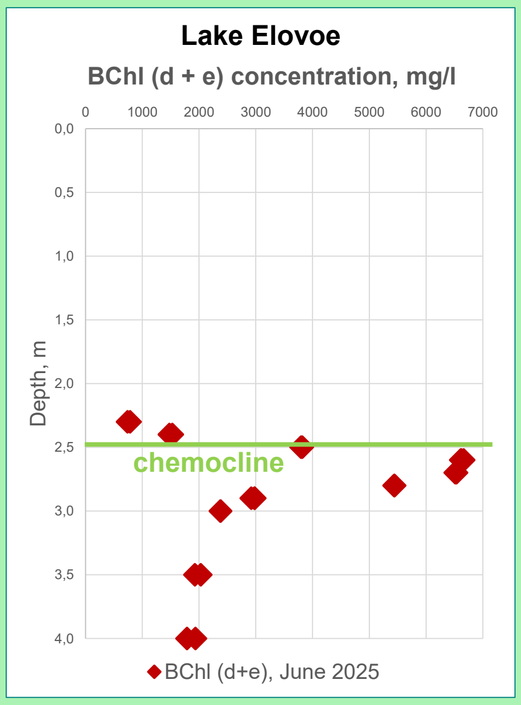


Figure 3. Depth distribution of Chl *a* and BChl (*d*+*e*) in two lakes.

Conclusions

Using spectrophotometric methods we analyzed main photosynthetic pigments (chlorophyll *a*, chlorosomal bacteriochlorophylls) in algal and bacterial cells in two meromictic lakes, Trekhtzvetnoe and Elovoe, found along the coastline of the White Sea.

Acknowledgments

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