# Underwater light quality and phototrophic microorganisms: what are the consequences of the ‘red shift’?

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## Underwater light climate of shallow lakes

The objective of this sub-project was to characterize the spectral changes of photosynthetically active radiation (PAR) within the water column in Hungarian shallow lakes with particular interest to humic and turbid waters. Systematic underwater light measurements were carried out in Lake Balaton in 2015, in Lake Fertő/Neusiedlersee in 2016 and in shallow turbid soda pans of the Danube-Tisza Interfluve in 2017 using a HR2000+ spectrometer (380 - 900 nm). Besides, relevant physical and chemical parameters – water temperature, Secchi-disk transparency, pH, conductivity, coloured dissolved organic matter (CDOM), total suspended solids (TSS) and dissolved oxygen concentration – were determined as well.

In case of Lake Balaton, five sampling stations were chosen for systematic underwater light measurements. According to the results obtained, the light climate varied remarkably along the longitudinal axis of the lake. In the Eastern basin, green/yellow light dominance (520-580 nm) was found, while in the Western basin, the underwater light climate was dominated by yellow/orange light dominance (570-620 nm). Light availability in the lake was predominantly affected by CDOM and TSS, however the abovementioned ‘red shift’ of the underwater light climate along the longitudinal axis depended only on CDOM levels (Eastern basin: 2.3-5.6 mg/L as Pt colour; Western basin: 11-36 mg/L as Pt colour).

In Lake Fertő/Neusiedlersee, two sampling stations were chosen: an open water sampling station (B0) and an inner lake sampling station (Kis-Herlakni). The open water sampling station was characterized by high inorganic turbidity (16-116 mg/L TSS), while the inner lake sampling station was marked by high CDOM concentration (120-180 mg/L as Pt colour). In the open water the dominant underwater light ranged from the yellow to the red (580-700 nm), while in the inner lake extreme red light dominance (670-690 nm) was observed and the near infrared radiation (> 800 nm) was relatively enriched.

Two pans with different character were chosen to represent soda lakes: a turbid one (Zab-szék pan) with high CDOM level (avg. ~ 500 mg/L as Pt colour) and extremely high TSS content (avg. ~ 2000 mg/L) and a brown-coloured one (Lake Sósér) with extremely high

CDOM (avg. ~ 6000 mg/L as Pt colour) and low TSS content (avg. ~ 150 mg/L). A pronounced 'red shift' of the underwater light climate was observed in both pans: the available light was dominated by red/deep red wavelengths the relative intensity of the infrared radiation showed also a remarkable increase. The brown-coloured pan, however, represented an even narrower light climate with deep red (670-690 nm) and infrared light dominance.

Summarizing the obtained results, intensity and spectral quality of the available light showed remarkable variability among the studied shallow lakes. In lakes having low CDOM concentration, aquatic microorganisms received relatively broad spectrum of available light with green, yellow or orange light dominance. Increasing CDOM concentration, however, resulted in a narrower spectrum and shifting the dominant wavelengths to the red, deep red or even to far-red regions.

## Relationship between underwater light quality and the composition of phototrophic communities

The objective of this sub-project was to characterize how the 'red shift' of the underwater light climate affects the composition and diversity of the phototrophic communities in Hungarian shallow lakes. In line with the underwater light measurements, qualitative and quantitative analysis of the phytoplankton was carried out with special respect to the photoautotrophic picoplankton community (with diameter shorter than 3 μm).

In Lake Balaton, phytoplankton biomass (chlorophyll *a* concentration) was higher in the Western (4-25 μg/L) than in the Eastern basin (1-6 μg/L) during the study period. Within the pico-size fraction, three distinct groups were distinguished: phycoerythrin-rich (PE-rich) picocyanobacteria, phycocyanin-rich (PC-rich) picocyanobacteria and picoeukaryotic green algae. Picoplankton abundance ranged from 0.1 to 3 x $10^5$ cells/mL in the Eastern basin, while significantly higher abundance values (0.3-5 x $10^5$ cells/mL) were found in the Western basin. The composition of the picoplankton was different in the Western and Eastern basin. In the Eastern basin, the picoplankton was dominated by PE-rich picocyanobacteria, while in the Western basin the contribution of this group was lower and the picoplankton was dominated by PC-rich picocyanobacteria during the productive period. In winter, picoeukaryotes were found in both basins, but their contribution to the total picoplankton biomass was higher in the western part of the lake as compared to the eastern part. The 'red shift' of the underwater light climate along the longitudinal axis of the lake, therefore, affected significantly the composition of the picoplankton.

In Lake Fertő, phytoplankton biomass was higher in the open water than in the inner lake, with chlorophyll *a* concentrations from 7 to 19 μg/L, and from 1.5 to 9 μg/L, respectively. Nano- and microphytoplankton composition was largely determined by the spectral characteristics of the underwater light climate. As a consequence of the relatively broad spectrum of available light in the open water, phytoplankton was more diverse there as compared to the inner lake. Also, picoplankton was more abundant in the open water than in the inner lake. In the open water, PC-rich picocyanobacteria were the dominant phototrophs through the whole year (with abundances from 8 x $10^4$ to 9 x $10^5$ cells/mL). In the inner lake, PC-rich picocyanobacteria was found with abundances between 0 and 2.6 x $10^4$ cells/mL from autumn to spring, while in winter, the picophytoplankton was dominated by eukaryotes (with abundances around $10^4$ cells/mL).

In regards of soda pans, phytoplankton biomass was much higher in the turbid pan as compared to the brown-coloured one, with chlorophyll *a* concentrations from 190 to 370 μg/L and from 3 to 25 μg/L, respectively. Phytoplankton was almost exclusively dominated by picoplankton whose composition was largely determined by spectral characteristics of the underwater light climate. In the turbid pan with red light predominance, PC-rich picocyanobacteria (with abundances from 2 x $10^6$ to 1 x $10^7$ cells/mL) and picoeukaryotic green algae (with abundances from 2 x $10^6$ to 2 x $10^7$ cells/mL) were the dominant groups, while in the brown-coloured one with underwater light spectrum confined to the deep red wavelenghts, picoeukaryotic green algae (abundance of up to 3 x $10^5$ cells/mL) prevailed over PC-rich picocyanobacteria.

Summarizing the obtained results, underwater light quality largely affected the composition of the phototrophic communities in the studied shallow lakes, especially within the pico-size range. As the orange light became the dominant light component under the water, PC-rich picocyanobacteria had competitive advantage over the PE-rich ones. With further 'red shift' of the light climate (deep red light dominance), however, picoeukaryotic green algae will prevail over the other two groups.

## Photoheterotrophic bacteria in shallow lakes

Aerobic anoxygenic phototrophs (AAP) have been detected by us for the very first time in Hungary. These bacteriochlorophyll *a*- producing bacteria use near-infrared radiation to generate metabolic energy and thought to be important players in oceanic carbon cycling. These microorganisms are barely studied in freshwaters, but their presence in the oxic layer has been justified in Central European mountain and North European humic lakes during the last years. Identification of AAP cells requires a specific method (Time-series observation based InfraRed Epifluorescence Microscopic (TIREM) protocol) and apparatus (epifluorescence microscope equipped with an infrared camera) as their detection is possible in the near-infrared region (800-900 nm) only. Our aim was to study the occurrence of AAP in Hungarian shallow lakes and to assess their importance in the microbial community (with special respect to the photoautotrophic picoplankton and heterotrophic bacterioplankton). As a result, AAP was found in high abundance in the studied lakes: their presence was verified in many Hungarian shallow lakes (e.g. Lake Balaton, Lake Fertő, in soda lakes of the Seewinkel, fishponds). The contribution of AAP to total bacterial abundance ranged between 4 and 40% which indicated the important role of these microorganisms in carbon cycling and energy flow. A pronounced seasonal dynamics was observed in Lake Balaton with high AAP abundances in summer and lower values in winter.

Moreover, the present study provided the first evidence for their presence in polyhumic soda pans, where the available light is low in visible range and enhanced in infrared radiation. As a consequence, the abundance of these photoheterotrophic bacteria was the highest ever found in aquatic environments: it ranged from $8 \times 10^5$ to $5 \times 10^7$ cells/mL in the turbid pan, and had even higher values (from $1 \times 10^6$ to $9 \times 10^7$ cells/mL) in the brown-coloured one. The reason of this latter might be the extremely high CDOM content combined with deep red/infrared light dominance in that environment. In summary, our results show niche differentiation along the red/infrared end of the light spectrum: picoeukaryotes overcome PC-rich picocyanobacteria in red/deep red light, followed by AAP dominance with the further advancement of the ‘red-shift’.

## Niche differentiation within the phototrophic community along the light spectrum

In order to characterize the light quality preference of different picoalgal taxa, one PE-rich picocyanobacterial strain, three PC-rich picocyanobacterial strains and three picoeukaryotic green algal strains were chosen (all belonging to different taxonomic groups). Before the experiments, a series of light intensities ranging from 10 to 60 µmol/m$^2$/sec was tested to determine the optimal light conditions. During the growth experiments, the cultures were grown at nine different wavebands (λ: 430, 460; 505, 525, 600, 610, 630, 660 and 690 nm) under light-limited condition (20 µmol/m$^2$/sec) at 24 °C.

According to our results, all of the studied isolates had different light quality preference. PE-rich picocyanobacteria were able to utilize green (505 and 525 nm), yellow/orange (600 and 610 nm) and red (660 nm) light efficiently, while their grown rates were lower under blue (430 and 460 nm), orange (630 nm) and deep red (690 nm) light. In contrast, PC-rich picocyanobacteria were not able to utilize either green (505 and 525 nm) or the blue (460 nm) light efficiently. Their growth rates were higher under blue (430 nm) and deep red (690 nm) light, however their most efficient light utilization was observed under orange (600, 610 and 630 nm) and red (660 nm) illumination. Growth rates of the picoeukaryotic green algae was the highest under blue (430, 460 nm) and red (660, 690 nm) illuminations. Green, yellow and orange (505, 525, 600, 610, 630 nm) lights were less appropriate for the growth of picoeukaryotes.

Different light utilization efficiency of the isolates led to their selective advantage at different light regions: for PE-rich picocyanobacteria the green and yellow light; for PC-rich picocyanobacteria the orange and red (630 nm) light and for picoeukaryotes the blue and the deep red light (690 nm) provided relative advantage. The pigment composition and ecophysiological characteristics of the unialgal cultures (green algal or PC-rich cyanobacterial isolates) also showed marked differences as a result of different illumination: for example, under deep red light, PC-rich cyanobacteria were characterized by limited energy transfer from phycobilisomes to photosystems and enriched carotenoid content. In contrast, similar negative effects in the ecophysiology of green algae were not observed.

Besides the unialgal experiments, niche-differentiation between picoeukaryotic green algae and PC-rich picocyanobacteria along the ‘red-end’ of the light spectrum was also confirmed using mixed cultures (1:1 abundance ratio at the beginning of the growing experiments). These cultures were grown under the same conditions (light, temperature) as the unialgal ones (see above). As a result, picoeukaryotic green algae prevailed over PC-rich picocyanobacteria under deep red light (690 nm), although from the yellow to the red wavebands (600 – 660 nm) the tendency was the opposite.

Comparing these results to the *in situ* underwater light climate measurements (see above), the underwater light environment in the Eastern basin of Lake Balaton with green/yellow light dominance (520-580 nm) is more advantageous for PE-rich picocyanobacteria, while the Western basin with yellow/orange light dominance (570-620 nm) is more beneficial for PC-rich picocyanobacteria. The yellow/red light dominance in browner lakes (e.g. open water and inner lake in Lake Fertő/Neusiedlersee, turbid soda pan) seemed to be adequate for both PC-rich picocyanobacteria and picoeukaryotes, while the exclusive deep red light dominance (670-690 nm) of the brown-coloured pan seemed to be especially advantageous for picoeukaryotes. This working hypothesis corresponds well with the obtained results on the composition of the picoplankton in the studied shallow lakes and pans.

Niche differentiation was also studied along the light spectrum using natural lake water communities: we conducted growth experiments under nine different monochromatic lights (see above) with samples originated from Lake Balaton, from a gravel pit lake, and also from several fishponds and reservoirs. Community composition at the beginning and at the end of the experiments was studied using an Utermöhl's inverted microscope. Despite initial differences, phytoplankton communities behaved similarly along the light spectrum. The blue region was usually beneficial for diatoms and chlorophytes, while the green and yellow region for diatoms and PE-rich picocyanobacteria (if they were present). A further red shift resulted in the dominance of PC-rich picocyanobacteria under orange and red (630 nm) illumination, however, under deep red light (690 nm), we found again an increase in the relative abundance of eukaryotic algae (mainly diatoms and chlorophytes). Our results have confirmed that the shift in the underwater light climate does considerably alter phytoplankton composition. The specific responses of various phytoplankton taxa to different light conditions might also be an important factor at future biotechnological applications.