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UV absorption reveals mycosporine-like amino acids (MAAs) in Tatra mountain lake phytoplankton*

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Abstract

Enhanced absorption of UV radiation, an effect characteristic of mycosporine-like amino acids (MAAs), is reported in samples of phytoplankton from six lakes in the Tatra Mountains National Park (Poland). It was demonstrated that the mass-specific UV absorption coefficients for the phytoplankton in these

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lakes increased with altitude above sea level. Based on a comparison with the phytoplankton of Alpine lakes, investigated earlier by other authors (cited in this paper), it may be inferred that the phytoplankton of Tatra mountain lakes produce MAAs, which protect plant cells from UV light, the intensity of which increases with altitude.

1. Introduction

Earlier studies by many authors demonstrated the significant influence of heightened ultraviolet (UV) radiation on plankton in high-altitude Alpine lakes (Cabrera et al. 1997, Halac et al. 1997, Vinebrooke & Leavitt 1999, Sommaruga & Garcia-Pichel 1999, Laurion et al. 2000, 2002, Tartarotti et al. 2004, Tartarotti & Sommaruga 2006). The greater intensity of UV radiation incident on high-mountain lakes in comparison with lowland water bodies is due to the shorter optical distance that solar radiation travels to reach mountain areas, and also to the generally better transparency of the atmosphere over mountains than over lowland areas. This increase in intensity is the greater, the shorter the wavelength of this radiation: for every 1000 m increase in altitude, the intensity for wavelength $\lambda = 370$ nm increases by 9%, for $\lambda = 320$ nm by 11% and for $\lambda = 300$ nm by 24% (Blumthaler et al. 1997). In addition, the waters of mountain lakes are generally more transparent to UV than lowland lakes, as they are less polluted by their surroundings and therefore contain less coloured dissolved organic matter (CDOM) and suspended particulate matter (SPM). Hence, UV radiation penetrates the waters of such lakes more effectively and to greater depths. In such a specific light climate, phytoplankton intensifies its activities to protect cells from damage and the photosynthetic process from photoinhibition. To this end, the cells of this phytoplankton produce appropriate protective pigments, including mycosporine-like amino acids (MAAs), as the cited papers have demonstrated. A measure, or at least an index, of the presence of MAAs in cells is their absorption coefficient in the relevant bands of the light spectrum utilized in all bio-optical studies of natural water bodies (Whitehead & Vernet 2000, Moisan & Mitchel 2001, Sinha et al. 2001, Laurion et al. 2002, Moisan et al. 2009). The occurrence of MAAs in mountain lakes in Europe has already been studied by Sommaruga & Garcia-Pichel (1999) and Laurion et al. (2000), among others, who demonstrated their presence in lakes in the Alps and the Pyrenees.

The objective of the present study was to determine whether the phytoplankton in the waters of a number of lakes in the Tatra Mountains (see Table 1, section 2) exhibit bio-optical properties similar to those in the Alpine lakes already investigated, and to ascertain the extent to which these properties differ from those of the phytoplankton in lowland Polish lakes and Baltic waters studied earlier by the authors and described in part by Ficek et al. (2012).

2. Material and methods

This preliminary bio-optical study was carried out in six lakes in the Tatra Mountains between 23 and 28 July 2012. The morphometric characteristics of these lakes are listed in Table 1 (after Choiński 2006). The diffuse attenuation coefficient of the downward PAR irradiance $K_{d,\mathrm{PAR}}$ was determined from in situ measurements of this irradiance in the euphotic layer with a HyperPro spectroradiometer (Satlantic) (PAR – Photosynthetically Active Radiation ∼400–700 nm). The Secchi depth z_{SD} was measured with a white disc 30 cm in diameter. For analysing the concentrations of optically active components (OACs – C_{SPM}, C_a, a_{CDOM}) and some of their properties, water samples were taken from an inflatable dinghy at several points on each lake from a depth of ca 20 cm below the surface directly into 5-litre dark plastic bottles. The water of three lakes was also sampled at four different depths, one below the other, using a bathometer. The samples were filtered two-three hours after sampling. Some of the filtered SPM to be used for measuring light absorption spectra and chlorophyll *a* concentrations was preserved in liquid nitrogen, while the remainder, for measuring dry mass, was stored in a freezer until analysis. In the laboratory, the light absorbed by the SPM in the 325–750 nm waveband was measured on a Hitachi F U-3900-H spectrophotometer equipped with an integrating sphere. The measurements of light absorption by phytoplankton are described in detail in Ficek et al. (2012) and Ficek (2013). They were performed twice. The first measurement, in transmission mode, yielded information on the attenuation of the light passing through the filter. The second one, in reflectance mode, determined the correction for backscattering from the filter. Following these two measurements, the filter was bleached (the pigments were destroyed) by saturating it with a 2% solution of CaClO; it took 5–15 minutes for the colours to disappear. Both measurements were then performed again. With the four spectra obtained in this way for each sample, the desired component coefficients of light absorption were determined using the relevant mathematical procedures described in Tassan & Ferrari (1995). Spectra of the light absorption by all SPM $a_{\mathrm{SPM}}(\lambda)$ and by non-phytoplankton particles $a_{\mathrm{NAP}}(\lambda)$ were thereby obtained. The difference between these two values yielded the absorption by phytoplankton: $a_{ph}(\lambda) = a_{\mathrm{SPM}}(\lambda) - a_{\mathrm{NAP}}(\lambda)$. It is the spectra of light absorbed by phytoplankton $a_{ph}(\lambda)$ that we shall be presenting in our graphs. The concentration of SPM $\mathrm{C_{SPM}}$ was determined by comparing

Table 1. Some morphometric characteristics of the six lakes in the Tatra Mountains National Park (after Choiński 2006). The 'abbreviations' column gives the short designations of the lakes used on the graphs

No.	Name of lake	Abbrevi-ations	Altitude of the lake surface above sea level [m]	Surface area of the lake [ha]	Maximum depth [m]	Mean depth [m]	Volume [thousand m^3]
1	Morskie Oko	MO	1392.8	34.9	51.8	28.4	9935.0
2	Czarny Staw nad Morskim Okiem	CS_MO	1579.5	20.6	77.0	37.6	7761.7
3	Wielki Staw	WS	1664.6	34.3	80.3	37.7	12967.0
4	Przedni Staw	PS	1668.3	7.7	34.6	14.6	1130.0
5	Czarny Staw	CS	1722.1	12.7	50.5	22.2	2825.8
6	Zadni Staw	ZS	1889.6	6.5	31.6	14.2	918.4

the mass of the dry Whatman filter GF/F (pore diameter ca 0.7 μm) before and after a known volume of water was passed through it. The chlorophyll *a* concentration in the water samples was determined using a standard spectrophotometric method (e.g. Jeffrey & Humphrey 1975). For analysing the light absorption spectra of CDOM $a_{\rm CDOM}(\lambda)$ the water samples were first passed through a Whatman GF/F glass microfibre filter (pore diameter ca 0.7 μm) and then through a Sartorius ACN membrane filter (pore diameter 0.2 μm). The filtrate was stored for about a week in the refrigerator at a temperature of 4°C. The light absorption spectra $a_{\rm CDOM}(\lambda)$ were determined with respect to twice-distilled water on a Hitachi U-3900-H spectrophotometer in 10 cm quartz cuvettes.

For comparison, water from a number of Mazurian lakes (Poland) and the Baltic Sea was analysed using the same technique.

Unfortunately we did not have the opportunity to directly measure the concentrations of these MAAs in our phytoplankton samples. But in the context of the papers cited above (e.g. Sinha et al. 2001, Moisan et al. 2009), the measured index of their content in these samples, i.e. the coefficient of UV light absorption, shows up the different concentrations in the phytoplankton of various lakes sufficiently well.

3. Results and discussion

Table 2 lists selected parameters of the samples of water taken from the Tatra mountain lakes.

Table 2. Parameters characterizing the optical properties of the surface waters of six lakes in the Tatra Mountains National Park

Lake	Chlorophyll a concentration C_a [mg m^{-3}]	Concentration of suspended particle matter C_{SPM} [g m^{-3}]	Volume absorption coefficient (concentration index) of coloured dissolwed organic matter (CDOM) a_{CDOM}(440 nm) [m^{-1}]	Diffuse attenuation coefficient of the downward PAR irradiance $K_{d,\,\text{PAR}}$ [m^{-1}]	Secchi depth z_{SD} [m]
Morskie Oko	1.25	0.48	0.12	0.18	11
Czarny Staw nad Morskim Okiem	0.60	0.39	0.023		
Wielki Staw	0.53	0.43	0.12	0.14	19
Przedni Staw	3.99	1.57	0.15	0.30	5
Czarny Staw	2.11	0.85	0.09		
Zadni Staw	1.07	0.53	0.25		

Figure 1 illustrates the light absorption spectra for phytoplankton living in the six Tatra mountain lakes. The spectra of the volume absorption coefficients for phytoplankton in water $a_{ph}(\lambda)$ depend both on the absorption properties of phytoplankton cells and on their concentration. Hence, to characterize the absorption properties of the phytoplankton cells alone (including their contents of particular pigments), one normally uses the specific absorption coefficient, which is referred to unit mass of chlorophyll a, i.e. $a^*_{ph}(\lambda) = a_{ph}(\lambda)/C_a$. The specific coefficient defined in this way no longer depends on the cell concentration in the water: its values and spectral distributions are determined by the absorption properties of the phytoplankton cells themselves, that is, they depend on the content and chemical/physical properties of the matter they consist of. The specific absorption coefficients $a^*_{ph}(\lambda)$ measured in phytoplankton from the six mountain lakes are illustrated in Figure 1b. This shows that the visible light absorption spectra for this phytoplankton have the two main absorption bands, typical of such spectra. The first of these bands – higher and broader, lying in the blue part of the spectrum (ca 440 nm) – is known as the Soret band and is due to absorption by almost all of the main pigments contained in phytoplankton cells (chlorophylls a, b, c and carotenoids). The second band – narrower and smaller, and placed in the red part (ca 665 nm) – is due primarily to the absorption of this light by chlorophyll a, and to a lesser extent by chlorophyll b, because of the small

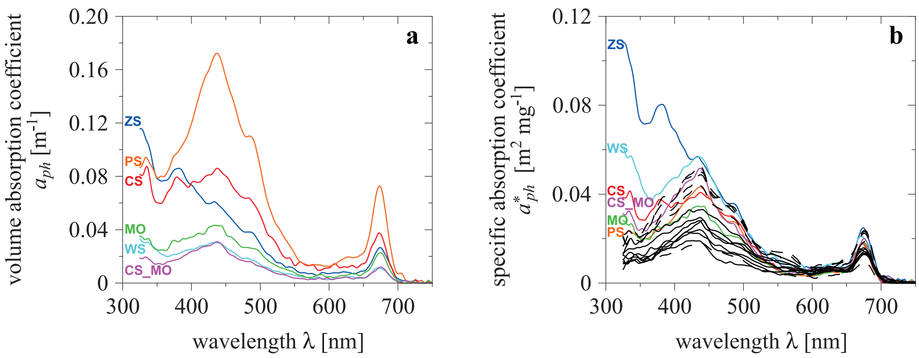

Figure 1. Light absorption spectra for phytoplankton measured in the surface waters of six lakes in the Tatra Mountains: a) – volume absorption coefficient a_{ph}, b) – specific absorption coefficients a^*_{ph}, referred to the chlorophyll a concentration in water. The abbreviations next to the curves on the plots denote the names of the lakes listed in Table 1. For comparison, Figure (b) also shows a group of typical spectra of specific absorption coefficients (black lines) that we measured using the same technique in lowland Mazurian lakes (Śniardwy, Mamry, Niegocin, Hańcza and Jeziorak) and in the Baltic Sea (black dashed lines)

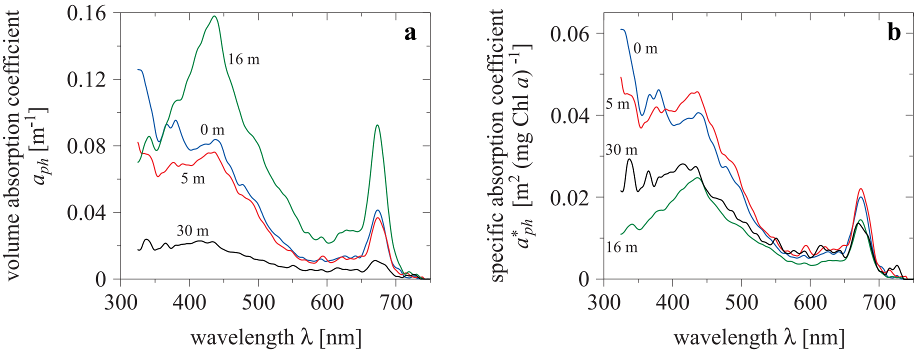

Figure 2. Spectra of light absorption coefficients measured at different depths in Lake Morskie Oko on 16.09.2011: a) volume absorption coefficients a_{ph}; b) specific absorption coefficients a^*_{ph}

concentration of the latter in phytoplankton. Absorption in the middle part of the visible spectrum is dominated by phycobilins and to a lesser extent by carotenoids (e.g. Woźniak & Dera 2007). In the spectra of $a^*_{ph}(\lambda)$ from the six Tatra mountain lakes the UV spectral range ($\lambda < 400$ nm) is particularly interesting (see Figure 1b). In this UV range the spectra of the specific absorption coefficients for phytoplankton $a^*_{ph}(\lambda)$ of lowland lakes and the Baltic Sea usually display much lower values (see the plots for the Mazurian lakes and the Baltic Sea in Figure 1b and, for example, Woźniak et al. (2011)). In contrast, the UV bands for the mountain lakes show distinct maxima of $a^*_{ph}(\lambda)$, in some cases even exceeding the maxima in the corresponding visible light spectrum. We see examples of this on the plots in Figure 1b for Lakes Zadni Staw (ZS), Wielki Staw (WS) and Czarny Staw (CS). Spectra containing such UV absorption maxima are known and have been found to be characteristic of organisms exposed to elevated levels of UV radiation (Sommaruga & Garcia-Pichel 1999, Sinha et al. 2001, Laurion et al. 2002, Moisan et al. 2009). This is the case in mountain lakes and is very evident in the surface waters of the Tatra mountain lakes we investigated; in deeper waters, to which less UV light penetrates, this effect is weaker (see Figure 2). Our measurements also show that the higher the altitude of a lake, where one would expect more UV in sunlight, the more UV is absorbed by the phytoplankton living in it (see Figure 3). The absorption of this UV in organisms exposed to elevated doses of this radiation is attributed to the group of pigments known as mycosporine-like amino acids (MAAs). These compounds have been identified in numerous aquatic organisms inhabiting environments exposed to high levels of UV radiation (Häder et al. 1998, Sommaruga 2001, Sinha & Häder 2002, Laurion et al. 2002, Tartarotti et al. 2004, Tartarotti & Sommaruga

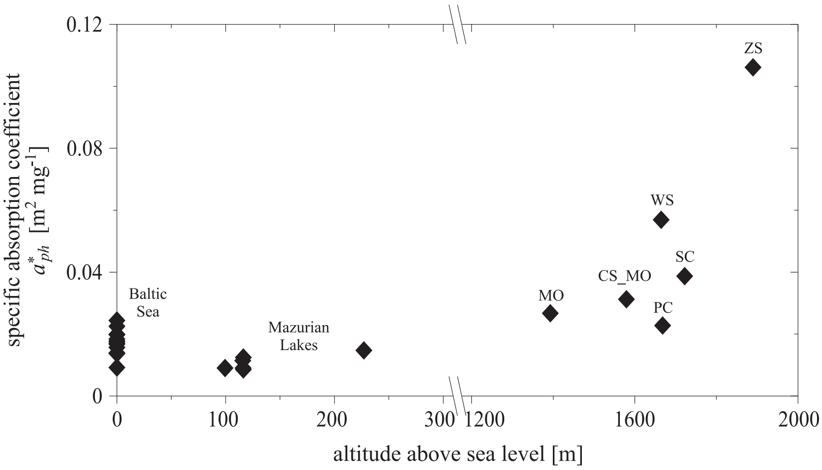

Figure 3. Dependence of specific absorption coefficients of UV radiation (in the 330 nm band) for surface water phytoplankton in lakes on their altitude above sea level for the six Tatra mountain lakes compared with typical values measured using the same technique in Mazurian lakes and the Baltic Sea

2006). MAAs play an important role in such organisms by preventing the destruction of their cells (Karentz 2001, Banaszak 2003, Moisan et al. 2009). According to these authors, the radiation absorption maxima for these compounds are located in the UV range ($\lambda_{\max} < 400$ nm): shinorine ($\lambda_{\max} = 334$ nm), mycosporine-glycine ($\lambda_{\max} = 310$ nm), porphyra-334 ($\lambda_{\max} = 334$ nm), palythinol ($\lambda_{\max} = 332$ nm), palythene ($\lambda_{\max} = 360$ nm), palythine ($\lambda_{\max} = 320$ nm) (Moisan et al. 2009). Figure 1 shows the absorption spectra for Tatra mountain lake phytoplankton and the maxima, which can be ascribed to absorption by MAAs.

The spectra measured at a number of different depths (Figures 2a, b) confirm the dependence of the UV radiation level on the absorption coefficients for phytoplankton. These plots show that the highest values of a^*_{ph} in the UV are recorded in surface waters and that they gradually decrease with increasing depths, to which less and less UV radiation penetrates.

The values of $a^*_{ph}(\lambda)$ measured in different lakes with respect to their altitude above sea level are compared in Figure 3. The configuration of points on this plot supports the hypothesis that the increase in UV ($\lambda <$ 400 nm) absorption is to some degree correlated with the altitude of a lake's water surface, above sea level, and hence with the intensity of UV radiation (see Introduction). But as this correlation is clearly governed by many other factors, our preliminary measurements do not constitute a basis for a quantitative description. In the example shown (Figure 3), only the results from Lake Przedni Staw (PS) deviate distinctly from this trend; the waters of this lake are eutrophic, as evidenced by the high level of chlorophyll a concentration, $C_a = 3.99$ mg m^{-3} (see Table 2). This is obviously due to anthropogenic factors: a mountain hut stands close by the banks of this lake. To a lesser extent this also applies to the Lake Czarny Staw (CS) ($C_a = 2.11$), for which the UV absorption is distinctly less than the trend shown on Figure 3.

The trend whereby concentrations of MAAs increase with the altitude of water bodies above sea level was also observed in zooplankton inhabiting lakes in Patagonia (Tartarotti et al. 2004).

4. Conclusion

The phytoplankton of Tatra mountain lakes, like the phytoplankton of some Alpine lakes investigated earlier, is characterized by a specific UV absorption typical of mycosporine-like amino acids (MAAs), which are produced in the cells of these organisms to protect them from the elevated doses of UV radiation to which mountain lakes are exposed. Such properties have not been observed in phytoplankton inhabiting the lowland Mazurian lakes or the Baltic Sea.

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