ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2004 Vol. 47, Št. 2: 57-64

Sprejeto (accepted): 2004-09-17

UV-B radiation screen and respiratory potential in phytoplankton in mountain lakes

Zaščita pred UV-B sevanjem in dihalni potencial fitoplanktona iz visokogorskih jezer

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Abstract. UV-B radiation screen and the respiratory potential (terminal electron transport system – ETS activity) were investigated in phytoplankton from five mountain lakes located on an elevation gradient from 1383 m to 2150 m a.s.l.. The amount of UV absorbing compounds in phytoplankton was generally higher on the lakes from higher elevation. The increased ETS activity of phytoplankton in higher lakes was suggested to reflect the energetic cost of generating the internal mechanisms for photoprotection.

Key words: mountain lakes, UV-B radiation, chlorophyll *a*, UV absorbing compounds, ETS activity, nutrient status

Abbreviations: DOC – dissolved organic matter, ETS – electron transport system, LK – Lake Krn, LKIn. – Lake Kriško Inferior, LKSup. – Lake Kriško Superior, LL – Lake Ledvica, LP – Lake Planina, UV – ultraviolet, UV AC – UV absorbing compounds.

Izvleček. Ugotavljali smo sposobnost za izgrajevanje UV zaščitnih snovi ter dihalni potencial (aktivnost terminalnega elektronskega transportnega sistema – ETS) fitoplanktona na petih visokogorskih jezerih, ki ležijo na različnih nadmorskih višinah, od 1383 do 2150 m. Vsebnost UV zaščitnih snovi je bila večja v fitoplanktonu iz višje ležečih jezer. Večja aktivnost ETS fitoplanktona iz višje ležečih jezer odraža potrebo po energiji, ki jo organizmi rabijo za mehanizme, s pomočjo katerih se zaščitijo pred sevanjem.

Ključne besede: visokogorska jezera, UV-B sevanje, klorofil *a*, UV absorbirajoče snovi, aktivnost ETS, obremenjenost s hranili

Okrajšave: DOC – raztopljena organska snov, ETS – elektronski transportni sistem, LK – Krnsko jezero, LKIn. – Spodnje Kriško jezero, LKSup. – Zgornje Kriško jezero, LL – Jezero v Ledvicah, LP – Jezero na Planini pri Jezeru, UV – ultravijolično, UV AC – UV absorbirajoče snovi.

Introduction

The increase of UV radiation at the Earth's surface, due to the gradual depletion of ozone in the atmosphere (WILLIAMSON 1995), has caused great concern about the consequent effects on terrestrial (BJÖRN 1999, GABERŠČIK & al. 2001, GABERŠČIK & al. 2002a) and aquatic ecosystems (HÄDER & al. 1998, GERM & al. 2002a,b). Mountain lakes are potentially vulnerable to climate changes due to their high elevation and, usually, their small size. Data on the dependence of intensity of UV-B on elevation differ. Increases of UV-B radiation (280-320 nm) are reported to range from 6-8% (CALDWELL & al. 1980) to 20% (BLUMTHALER & al. 1993) per 1,000 m of elevation. Higher lakes usually contain lower concentrations of DOC which is considered to be the important factor controlling the penetration of UV into water (HUOVINEN & al. 2003). Organisms in higher and cleaner lakes are therefore exposed to higher UV-B doses, usually without any refuge from damaging solar radiation (WILLIAMSON 1995). UV-B radiation affects many biological and physiological processes in primary producers (HÄDER & al. 1998, ROZEMA & al. 1997). Experimental studies show that species and populations originating from naturally high UV-B locations i.e. from high elevations or low latitudes are less sensitive to UV-B radiation than those from low UV-B locations (SULLIVAN & al. 1992, VILLAFAÑE & AL. 1999). Protection against direct and indirect influences of UV-B is most important in photosynthetic organisms that depend on solar radiation as the primary source of energy in their natural environment (HESSEN & al. 1995). One of the important responses of phytoplankton to UV is the synthesis of UV absorbing compounds, such as mycosporine-like amino acids (MAAs), which protect the cells by preventing UV radiation from reaching and damaging vital molecules such as nucleic acids, especially DNA (HÄDER & al. 1998, BJÖRN 1999, GABERŠČIK & al. 2002a, GERM & al. 2002a). There is an increased need for energy during stress (AMTHOR 1995, GERM & GABERŠČIK 2003) since the establishment of protective mechanisms i.e. synthesis of component materials demand additional supplies of energy from the respiratory process (GULMON & MOONEY 1986).

In this study we have estimated the respiratory potential, and the ability of phytoplankton from mountain lakes located on an elevation gradient, to produce UV screening substances.

Materials and methods

The study was carried out during summer 2001 and 2002 in the Julian Alps in NW Slovenia. The lakes differ in elevation, trophic status, dimensions and type of activities in the watershed. Their characteristics are listed in Table 1.

Chlorophyll (Chl.) a: samples of water were taken with a Van Dorn sampling device (Wildco Ltd., USA) from the boat. 0.5 to 3 litres of water at 2.5 m intervals from the lake surface to the bottom at the deepest point of the lakes were collected around noon and immediately filtered through Whatman GF/F filters and frozen. The samples were homogenised in 2 ml of extraction solution (90% (v/v) acetone) and centrifuged at 8500 g, 4°C, for 4 min in a top refrigerated ultracentrifuge (2K15, Sigma, Osterode, Germany) and the absorbance of the supernatant measured. The equation of JEFFREY & HUMPHREY (1975) was used to calculate the concentration of chl. *a*.

UV absorbing compounds: the basic procedure used for extracting UV screening substances follows the method of CALDWELL (1968). The frozen samples (see above) were homogenised in 5 ml extraction solution containing methanol : distilled water : HCl (37% v/v) (79:20:1 v/v/v), incubated for 20 minutes and centrifuged (1600 g, 10°C, 10 min) in a top refrigerated ultracentrifuge. The absorbance of the supernatants was measured from 280 to 400 nm at intervals of 1 nm. The relative amounts of UV absorbing compounds were determined by integrating the values of absorbance and normalised to the concentration of chl. a.

Table 1. Key characteristics of studied lakes. Geographical characteristics are listed according to DOBRAVEC AND ŠIŠKO (2002).
Tabela 1. Osnovne značilnosti preučevanih jezer. Geografske značilnosti so povezete po DOBRAVEC IN ŠIŠKO (2002).

Lake	Kriško Sup.	Kriško In.	Ledvica	Planina	Krn
Geogr. position	N 46°24'32''	N 46°23'59''	N 46°20'25''	N 46°18'40''	N 46°17'09''
	E 13°48'34''	E 13°48'24''	E 13°47'12''	E 13°49'56''	E 13°41'08''
Altitude	2150	1880	1830	1430	1383
Surface	0.662	0.862	2.187	1.562	4.534
Depth	9	9	15	11	18
Total P	14.07	11.38	21.24	111.66	19.4
Total N	1.75	1.83	2.03	2.65	1.86
chl. *a*	0.85	0.57	0.37	14.31	6.36
Temperature	7.5	9.8	6.6	9.0	10.8
Secchi disc	bottom	bottom	bottom	bottom	bottom
Max penet.UV-B	9	8	7	1.5	5
Trop. state	oligotrophic	oligotrophic	oligotrophic	hypereutrophic	eutrophic

Legend: Geogr. position (geographical position), altitude (m), surface (ha), depth (m), total P (total phosphorus, µg/l), total N (total nitrogen, mg/l), chl. *a* (chlorophyll *a*, µg/l), temperature (°C), Secchi disc (m), max. penet. UV-B (maximum penetration of UV-B, m), trop. state (trophic state).
Legenda: Geogr. position - geografska lega, altitude - nadmorska višina (m), surface - površina (ha), depth - globina (m), total P - celoten fosfor (µg/l), total N - celoten dušik (mg/l), chl. *a* - klorofil *a* (µg/l), temperature - temperatura (°C), Secchi disc - Sekijeva plošča (m), max. penet. UV-B - maksimalna globina prodiranja UV-B (m), trop. state - trofično stanje.

Terminal electron transport system activity: the terminal electron transport system (ETS) activity of mitochondria was measured as described by PACKARD (1971). Samples of water taken from the vertical profile, were filtered through 100 µm mesh size to remove zooplankton, and then through Whatman GF/F filters. Samples were homogenised in 4 ml of ice-cold buffer in a mortar followed by an ultrasound homogenizer (4710; Cole-Parmer, Vernon Hills, IL, USA) at 40W and centrifuged (8500 g, 2°C, 4 min) in a top refrigerated ultracentrifuge. The 0.5 ml of supernatant was mixed with 1.5 ml of substrate solution and 0.5 ml of iodo-nitro-tetrazolium-chloride (INT) solution and incubated for 40 minutes at room temperature. During incubation the INT was reduced to formazan. The absorption of formazan was measured at 490 nm. ETS activity was calculated from the rate of INT reduction, which was converted to the equivalent amount of oxygen (KENNER & AHMED 1975), and normalised to the concentration of chl. *a*.

Chemical analyses: samples of water were taken as stated above. The contents of total phosphorus (Valderrama 1981) and total nitrogen (A.P.H.A. 1998) were measured spectrophotometrically.

Results and discussion

The trophic levels of the lakes varied with the elevation; the lakes above 1800 m were oligotrophic while those at lower altitudes were eutrophic to hypereutrophic. Average of total phosphorus, total nitrogen and concentration of chl. *a*, as well as temperature in water column were measured in year 2001 and 2002 (Tab. 1). The correlation between ETS activity and concentration of phosphorus was $r=0.98$ ($p<0.05$), in agreement with *del Giorgio* (1992). Factors like latitude, altitude, land use, vegetation cover and geological composition of watershed can strongly influence the level of incident radiation and the optical properties of the water column (KARENTZ & al. 1994). Clean water ecosystems in high elevation mountains, may receive high doses of UV-B light at significant depths (HESSEN & al. 1995). The maximum penetration depth of UV-B was significantly negatively correlated to the content of chl. *a* ($r=-0.94$, $p<0.05$). The maximum penetration depth of UV-B radiation was highest in Lake Kriško Sup., and lowest in Lake Planina. Depth of maximum penetration reflects the DOC concentrations and the presence of planktonic organisms (NIELSEN 1996). Hodoki & Watanabe (1998) also proved, that attenuation coefficient for UV-A (320-400 nm) and UV-B (280-320 nm) was highly correlated with the concentration of chl. *a*. The amount of chl. *a* in lake water was highest in the hypereutrophic Lake Planina (Tab. 1). The highest concentration of chl. *a* was observed to be close to the bottom in most of the lakes. This has been reported for other high-mountain lakes (SOMMARUGA & GARCIA-PICHEL 1999). TÔTH & al. (1995) suggested that the photosynthetically most active zone had been forced deeper in the lake due to UV-B.

SOMMARUGA & GARCIA-PICHEL (1999) reported about the maximum amount of UV absorbing compounds (normalised to the concentration of chl. *a*) close to the bottom. That was not the case in all studied lakes (Fig. 1). The bioaccumulation of UV absorbing compounds in copepods has been suggested to be the reason for the observed gradient (SOMMARUGA & GARCIA-PICHEL 1999). VILLAFAÑE & al. (1999) also observed a high rate of bioaccumulation of these compounds in the food web. The possible reason for the concentration of UV absorbing compounds reaching a maximum near the bottom was probably the sedimentation of phytoplankton, coupled with the fact that UV absorbing compounds were chemically more stable than chl. *a*. Phytoplankton from transparent, high elevation and oligotrophic lakes generally contained more UV absorbing compounds than specimens from lower elevation lakes (Fig. 1). These results showed that the production of UV absorbing compounds in primary producers in the high elevation lakes was likely a response to the higher flux of UV-B. The frequently observed correlation between UV-B radiation and the concentration of UV absorbing compounds in many primary producers shows that these substances protect vulnerable targets in organisms (SOMMARUGA & GARCIA-PICHEL 1999, GABERŠČIK & AL. 2002A, GERM & AL. 2002A, WINKEL-SHIRLEY 2002).

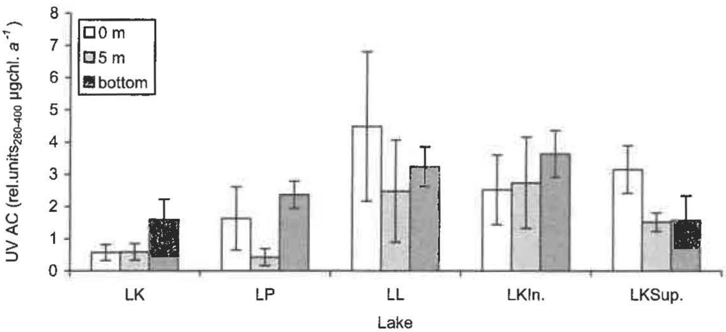

Fig. 1. Amount of UV absorbing compounds (normalised) in phytoplankton on the lake surface (white bars), 5 m deep (light grey bars), and bottom (dark grey bars) (n=3, mean±SD).
Sl. 1. Vsebnost UV absorbirajočih snovi (normirano) v fitoplanktonu na površini (beli stolpci), na 5 metrih (svetlo sivi stolpci) in na dnu (temno sivi stolpci) (n=3, povprečje±SD).

The synthesis of secondary substances is energetically costly and they are produced if the damage due to UV-B is bigger than metabolic costs for production (GABERŠČIK & al. 2002b). ETS activity is a measure of the metabolic potential of organisms. ETS activity per volume of water was higher in lower, eutrophic lakes (Fig. 2), which had high concentration of chl. *a* (Tab. 1). Several investigations (CHO & AZAM 1990, DEL GIORGIO 1992) showed a significant correlation between ETS and chl. *a* in a wide range of lakes. On the other hand, ETS activity (normalised to the concentration of chl. *a*) was higher in the pytoplankton from more oligotrophic lakes receiving higher UV-B doses (Fig. 3). It has already been reported, that ETS activity of pytoplankton increased under enhanced UV-B radiation (FERREYRA & al. 1997, GABERŠČIK & al. 2002a, GERM & al. 2002a). This can be explained by the increased need for energy, for mechanisms involved in photoprotection, i.e. production of UV absorbing compounds.

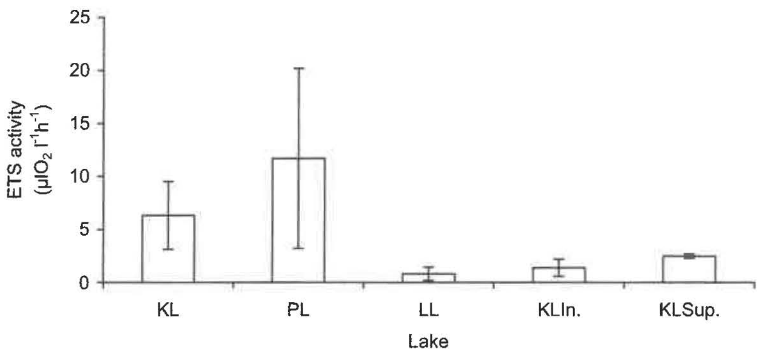

Fig. 2. ETS activity ($\mu lO_2/l/h$) in phytoplankton in vertical profile (n=6-9, mean±SD).
Sl. 2. Aktivnost ETS ($\mu lO_2/l/h$) v fitoplanktonu na vertikalnem profilu (n=6-9, povprečje±SD).

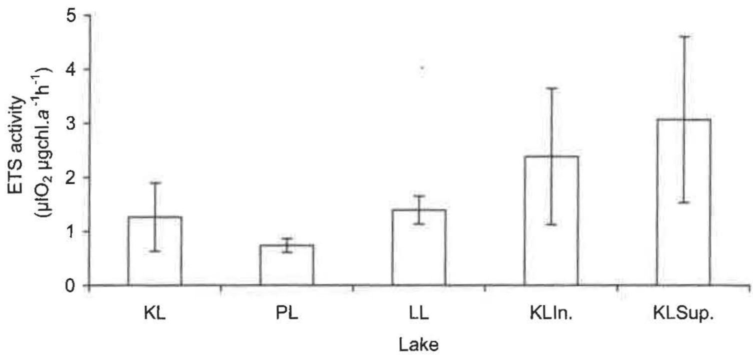

Fig. 3. ETS activity (normalised) in phytoplankton in vertical profile (n=6-9, mean±SD).
Sl. 3. Aktivnost ETS (normirano) v fitoplanktonu na vertikalnem profilu (n=6-9, povprečje ±SD).

In conclusions, the present study reveals, that phytoplankton from high mountain and olygotrophic lakes protected their vulnerable targets in the cells by synthesis of UV screening substances. This process required additional energy, derived from respiratory potential.

Povzetek

Naraščanje UV sevanja na Zemeljski površini, ki je posledica tanjšanja ozonske plasti v atmosferi, je predmet mnogih raziskav. Čeprav je delež UV-B sevanja v sončnem sevanju majhen, je njegov vpliv na primarne proizvajalce velik. Visokogorska jezera so še posebej občutljivi sistemi na klimatske spremembe. Po nekaterih podatkih se UV-B sevanje (280-320 nm) na vsakih 1000 m nadmorske višine poveča za 6-8%, oz. za 20%.

Visokogorska jezera vsebujejo navadno nizke koncentracije raztopljenih organskih snovi, ki v veliki meri vplivajo na prodiranje UV sevanja v vodo. Organizmi v visokogorskih in čistih jezerih so tako izpostavljeni višjim odmerkom UV-B sevanja brez možnega zatočišča pred škodljivimi žarki. UV-B sevanje vpliva na mnoge biološke in fiziološke procese pri primarnih proizvajalcih.

Ugotavljali smo sposobnost fitoplanktona za izgrajevanje UV zaščitnih snovi ter dihalni potencial (aktivnost terminalnega elektronskega transportnega sistema – ETS) na Jezeru na Planini pri Jezeru, Krnskem jezeru, Jezeru v Ledvicah ter Spodnjem in Zgornjem Kriškemu jezeru, ki ležijo na različnih nadmorskih višinah, od 1383 do 2150 m. Vsebnost UV zaščitnih snovi je bila večja v fitoplanktonu iz višje ležečih jezer. Aktivnost ETS (normalizirana) je bila večja v Jezeru v Ledvicah ter obeh Kriških jezerih. Večja ETS aktivnost fitoplanktona iz višje ležečih jezer odraža potrebo po energiji, ki jo organizmi rabijo za mehanizme, s pomočjo katerih se zaščitijo pred sevanjem.

Acknowledgements

This research was a part of SLO Alpe2 (3311-01-2183388) financed by Ministry of Education, Science and Sport of Republic Slovenia. The financial support is gratefully acknowledged. Author is grateful to Dr. Roger H. Pain for correction of the paper.

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