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Combined effects of light and temperature on growth, photosynthesis, and pigment content in the mat-forming cyanobacterium *Geitlerinema amphibium*

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Abstract

Geitlerinema amphibium (BA-13), mat-forming cyanobacterium from the southern Baltic Sea, was grown at three irradiances [5, 65, and 125 μmol(photon) m^{-2} s^{-1}] and three temperatures (15, 22.5, and 30°C). To determine the effect of the investigated factors and their interaction on culture concentration, pigment content, and photosynthetic parameters of cyanobacterium, factorial experiments and two-way analysis of variance (*ANOVA*) were carried out. Both chlorophyll (Chl) *a* and phycobilins (PB) were influenced by the irradiance and temperature, but stronger effect was noted in the case of the former one. Chl *a* and PB concentration per 100 μm of filament dropped above 4-fold with the increasing irradiance. The ratios between individual carotenoids [*β*-carotene, zeaxanthin, and myxoxanthophyll (Myx)] and Chl *a* increased significantly with an increase in the irradiance. The greatest fluctuations were observed in the ratio of Myx to Chl *a* (above 10-fold). Thus, Myx was suggested as the main photoprotective carotenoid in *G. amphibium*. Based on photosynthetic light response (PI) curves, two mechanisms of photoacclimation in *G. amphibium* were recognized: a change of photosynthetic units (PSU) number and a change of PSU size. These two mechanisms constituted the base of significant changes in photosynthetic rate and its parameters, such as the compensation point (P_C), the initial slope of photosynthetic curve (α), saturation irradiance (E_K), maximal photosynthetic rate (P_{max}), and dark respiration rate (R_D). The greatest changes were observed in P_C values (about 15-fold within the range of the factors tested). Studied parameters showed a wide range of changes, which might indicate *G. amphibium* ability to acclimatize well to irradiance and temperature, and indirectly might explain the successful growth of cyanobacterium in dynamically changing environmental conditions.

Additional key words: *Geitlerinema amphibium*; irradiance; microbial mats; photosynthesis; pigments; temperature.

Introduction

Microbial mats in the Puck Bay (the Southern Baltic, Poland) are mainly formed at a depth below 1 m, on a medium- and fine-grained sand. They are composed of unicellular and filamentous microorganisms consolidating the surface of a deposit, protecting it against deteriorating influence of waves and currents. Coccoid forms of Chroococcales and filamentous forms of Oscillatoriales and Nostocales predominate in the mat composition in the Puck Bay (Witkowski 1986). Oscillatoriales absolutely outnumber other filamentous cyanobacteria species. Coccoid cyanobacteria dominate in the spring, whereas filamentous forms appear in the summer. *Geitlerinema amphibium* Anagnostidis 1989, as a permanent element of summer microbial mats in the Puck Bay (Witkowski 1986), is an example of organisms using their outstanding acclimation ability in the best possible way.

Irradiance and temperature are basic factors affecting

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Abbreviations: *ANOVA* – analysis of variance; AP – allophycocyanin; CCBA – Culture Collection of Baltic Algae; Chl – chlorophyll; DM – dry mass; E_K – saturation irradiance; Myx – myxoxanthophyll; N – number of filament units; OD – optical density; P_C – compensation point; PB – phycobilins; PC – phycocyanin; PE – phycoerythrin; PI – photosynthetic light response; P_{max} – maximum photosynthetic rate; P_N – net photosynthetic rate; PSII – photosystem II; PSU – photosynthetic units; R_D – dark respiration rate; RP-HPLC – reversed-phase high performance liquid chromatography; α – initial slope of photosynthetic curve.
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photoautotrophic life. They influence metabolic processes, and consequently a growth rate, photosynthetic activity, and pigment composition of cells. Microbial mat cyanobacteria demonstrate a great adaptability in response to high fluctuations of environmental factors. These organisms may be covered periodically by a layer of sediment, which limits availability of light. On the other hand, sediment dispersion, caused by waves and currents, can lead to a rise in light intensity. In the coastal water of the Puck Bay, which corresponds to the fifth type of waters (Jerlov 1976), irradiance is reduced to about 50% of the surface irradiance at 1 m depth (Dring 1998). Thus, the light intensity could reach values of about 500 μmol(photon) m^{-2} s^{-1} at that depth, but the real light intensity has often lower values, reaching the organism in a mat about 5 mm thick, and it can fluctuate. Such rapidly changing environmental factors forced microbial mat organisms to develop many acclimation mechanisms to minimize the stress of extremely changeable light intensity. High irradiance may damage PSII reaction centre by photooxidation. However, some carotenoid pigments may provide effective protection against such disadvantageous influence of light (Hirschberg and Chamovitz 1994, Steiger *et al.* 1999, Lakatos *et al.* 2001, MacIntyre *et al.* 2002). Photosynthetic organisms respond to decreased light intensity by increasing the size or/and the number of PSU, which may be reflected in characteristic patterns of PI curves (Platt *et al.* 1980, Prézelin 1981, Ramus 1981, Richardson *et al.* 1983, Henley 1993, Dring 1998, Mouget *et al.* 1999, MacIntyre *et al.* 2002). Variation in α and P_{max} (expressed per biomass or per Chl *a* unit) plays a key part in interpreting physiological responses to changes in environmental conditions. In order to determine the interactions between light and temperature, the effect of both factors on growth, pigment content, and photosynthesis of cyanobacteria should be examined together (Ibelings 1996, MacIntyre *et al.* 2002, Defew *et al.* 2004).

In earlier studies, the influence of light intensity [20–170 μmol(photon) m^{-2} s^{-1}] and temperature (20–35°C) on the growth of *G. amphibium* was described (Latała and Misiewicz 2000). Its growth was saturated at 120–170 μmol(photon) m^{-2} s^{-1} and depended on the temperature. A high increment of cyanobacterial biomass was observed at 120 μmol(photon) m^{-2} s^{-1} and 35°C, as well as 170 μmol(photon) m^{-2} s^{-1} and 30°C, while interaction between the highest temperature (35°C) and the highest light intensity (170 μmol(photon) m^{-2} s^{-1}) inhibited the growth. Results of this work encouraged us to widen the range of examined factors to describe the influence of lower light intensity and temperature on the growth of *G. amphibium*. These additional data enabled the authors to fulfil the main objective of this paper, which was to recognize and indentify cyanobacterial mechanisms of photoacclimation by determining changes in content of Chl, carotenoids, and PB, and characterization of photosynthesis by PI curves. Such information is very useful to understand, why Oscillatoriales dominate in the summer microbial mats.

Materials and methods

Organism and culture condition: Cyanobacterium, *G. amphibium* (BA-13), was isolated from microbial mats of microphytobenthos in the coastal part of the Puck Bay near Władysławowo (the Southern Baltic, Poland) (Latała and Misiewicz 2000). This strain (BA-13) is maintained as unialgal culture in CCBA (http://www.ocean.univ.gda.pl/~ccba/) at the Institute of Oceanography, University of Gdańsk, Poland (Latała *et al.* 2006). Batch cultures were carried out in 150 ml of sterilized BG-11 medium (Stanier *et al.* 1971) in 300 ml glass Erlenmeyer flasks. These media were prepared from the Baltic water of salinity 8 ppt. Experiments were carried out under the following combinations of irradiance and temperature levels:

(*1*) Irradiance: 5, 65, 125 μmol(photon) m^{-2} s^{-1}. The intensity of light was measured using a quantum-meter (*LI-189, LI-COR Inc.*, Nebraska, USA) with a cosine collector. The strain culture was incubated under a 16/8 h light/dark cycle. Fluorescent lamps (*Cool white 40W, Sylvania*, USA) were used as source of the irradiance. To obtain more intensive light, they were combined with halogen lamps (*100W, Sylvania*, USA). Light intensity was controlled by using neutral density filters.

(*2*) Temperature: 15, 22.5, 30°C. Experiments were conducted in small incubators capable of maintaining constant temperature condition (± 1°C).

Cultures were acclimated to every combination of factors for 7 d. Then they served as inoculum for test cultures, where the initial number of filament units was 1,000 ml^{-1} (1 filament unit = 100 μm). Similarly as in the Baltic Monitoring Programme, a filament of 100 μm length was accepted as the filament unit (Kononen 1992). Test cultures were performed in 4 replicates and were incubated for 14 d at each combination of light and temperature. During the incubation, the cultures of cyanobacteria were optically thin enough, thus, all filaments were exposed to a comparable irradiance. On the last day of the incubation, during the exponential growth phase, the culture concentration, pigment content, and photosynthetic activity were measured for each replicate.

Culture concentration: The culture concentration was represented N, DM, and OD. N was determined by light microscope (*Amplival, Carl Zeiss,* Jena, Germany*)* in Fuchs-Rosenthal chamber, and OD was measured spectrophotometrically at 750 nm with *DU530 UV-VIS Life Science* spectrophotometer (*Beckman*, USA) in a 1-cm glass cuvette. These data provided the basis for

determining the value of the correlation coefficient (r = 0.982) and linear correlation (y [filament unit ml^{-1}] = 70.66 10^{-5} x – 87.10^{-3}; where y = N per ml, and x = OD) (Latała and Misiewicz 2000). Ns in test cultures was determined optically in each culture and then derived from the calibration curve mentioned above. Samples for DM estimation were filtered through *Whatman GF/C* glass fibre filters, dried at 60°C for 24 h and weighted with the accuracy of 10^{-1} mg.

Pigment analysis: RP-HPLC method was used to analyse Chl and carotenoid pigments. Procedures of pigment extraction and separation were the same as described by Jodłowska and Latała (2003). Pigments were identified by comparison of both retention times and absorption spectra with those of pigment standards. High purity pigment standards (Myx, zeaxathine, β-carotene, and Chl *a*) were purchased from the International Agency for ^{14}C Determination VKI in Denmark.

PB were extracted according to Stewart and Farmer (1984). Each filter was thoroughly homogenized in a medium consisting of 0.25 M Trizma base, 10 mM disodium EDTA, and 2 mg ml^{-1} lysozyme. Medium pH was adjusted to 5.0 with HCl. Homogenates were incubated in darkness for 2 h at 37°C and then for 20 h at 2°C. To remove cell debris and filter particles, the pigment extract was centrifuged at 2,124 × *g* for 5–10 min. The absorbance of the pigment extract was measured at 565, 620, 650, and 750 nm with *DU530 UV-VIS Life Science* spectrophotometer (*Beckman,* USA) in a 1-cm glass cuvette. PB concentration was calculated according to Tandeau de Marsac and Houmard (1988): PC [mg ml^{-1}] = $[(E_{620} - E_{750}) - 0.7\ (E_{650} - E_{750})]\ 7.38^{-1}\ V_a\ V_b^{-1}$, PE [mg ml^{-1}] = $[(E_{565} - E_{750}) - 2.8\ PC - 1.34\ AP]\ 12.7^{-1}\ V_a\ V_b^{-1}$, and AP [mg ml^{-1}] = $[(E_{650} - E_{750}) - 0.19\ (E_{620} - E_{750})]\ 5.65^{-1}\ V_a\ V_b^{-1}$, where: V_a = extract volume [ml], V_b = sample volume [ml], and E_x = extinction (absorption) measured at wavelength x in a 1-cm cuvette.

PI curves: Oxygen exchange was measured by a volumetric microrespirometer (*the Franciszek Górski Department of Plant Physiology PAS*, Cracow, Poland), composed of 5 microchambers connected individually to capillaries. Four chambers contained cyanobacterial samples, whereas the fifth one was used as a control for air pressure changes. A suspended drop of KOH was added to each chamber, therefore changes in the gas volume were caused only by oxygen production or consumption by cyanobacterium (for detailed description of microrespirometer *see* Zurzycki and Starzecki 1971). To estimate PI curves, cyanobacterial samples were always taken from the end of the dark phase of the light/dark cycle. Dark respiration rate measurements were done first after darkening the measuring chamber. For each culture condition, oxygen production was determined at eight light levels: 4, 8, 18, 29, 61, 157, 353, and 960 μmol(photon) $m^{-2}\ s^{-1}$. Light intensity was controlled by neutral density filters, the irradiance was measured using a quantum-meter with a cosine collector (*LI-189, LI-COR Inc.*, Nebraska, USA). At each irradiance, the samples were illuminated for about 10 min. The course of PI curves was fitted to the data using the *STATISTICA®6.0* software program and mathematical function of Platt and Jassby (1976). On the basis of these data the following photosynthetic parameters were estimated: P_C, E_K, α, P_{max}, and R_D.

Factorial experiments and statistical analysis: To determine the effect of investigated factors and their interaction on the culture concentration, pigment content, and photosynthetic parameters of *G. amphibium*, factorial experiments were carried out. Two-way analysis of variance, *ANOVA*, was used to assess the main effects and their interaction (Brzeziński and Stachowski 1981). In a factorial experiment method, values of the independent variables occurred at the same intervals and a replicate number was always the same. In our investigations, all experimental variants were run in 4 replicates. It enabled calculations by creating mutually orthogonal polynomials. Polynomial fitting by the method ξ' (ksi prime) (Snedecor and Cochran 1980, Oktaba 1986) was simplified by the use of tables of orthogonal polynomials (Fisher and Yates 1963). The method made possible to determine the influence of investigated factors and their interaction on measured parameters by calculation of a regression equation. The *STATISTICA®6.0* program was used to find the best regression equation and the results have been presented graphically as response surface form using the *Golden Software Surfer 8.0* program.

Results

***G. amphibium* culture concentration**: Factorial experiments showed that both irradiance and temperature had the promoting effect on the cyanobacterial culture concentration (Fig. 1). Maximal values of DM (about 0.55 mg per ml) were noted at the light intensities of 95–125 μmol(photon) $m^{-2}\ s^{-1}$ and temperatures of 22–30ºC, and they were almost 10-fold higher than minimal ones, obtained at about 15ºC within the whole range of light intensities tested (about 0.05 mg ml^{-1}).

Results of variance analysis showed that the influence of the temperature on culture concentration was higher than the influence of the irradiance and the interaction of both factors (Table 1). About 44% of total sum of squares was accounted for by temperature sum of squares,

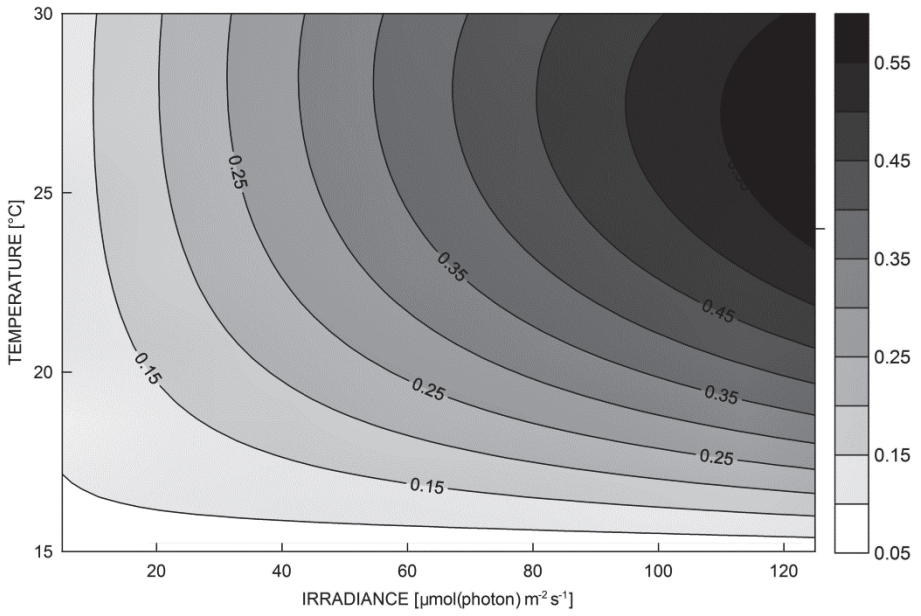

Fig. 1. Response-surface estimation of *G. amphibium* dry mass [mg per ml of culture] at 14th d of cultivation at different temperatures and irradiance levels.

whereas 35% and 21% were accounted for the irradiance and the interaction of both investigated factors, respectively.

Photosynthetic pigments: There were 4 pigments identified in *G. amphibium*: myxoxanthophyll, zeaxanthin, Chl *a*, and *β*-carotene. Chl *a* content, expressed in pg per filament unit (Fig. 2), dropped significantly with the increasing light intensity (the mean difference, calculated for the whole range of temperature, was about 4.5-fold), but it increased slightly with the increasing temperature (the mean difference, calculated for the whole range of light intensity, was merely about 1.3-fold). The lowest values noted within the range of 70–125 µmol(photon) m^{-2} s^{-1} and the low temperature of 15–17°C (~0.2 pg filament $unit^{-1}$) were only 17% of the highest values at the low light intensity of 5–10 µmol(photon) m^{-2} s^{-1} and high temperature of 25–30°C (~1.2 pg filament $unit^{-1}$). Based on the data of variance analysis, Chl *a* content was influenced almost entirely by the irradiance (Table 1). About 96% of total sum of squares was accounted for by irradiance sum of squares, while the influence of the temperature and the interaction of both investigated factors were very low (2.6% and 1.3%, respectively), but statistically significant at p=0.05.

Effects of the irradiance as well as temperature and the interaction between the factors investigated were statistically insignificant (p>0.05) on the total sum of carotenoids in cyanobacterial filament unit. The filament concentration of carotenoids remained constant within the whole range of the light intensity and temperature (~0.33 pg filament $unit^{-1}$). However, the concentration of the individual carotenoids in one filament, except for zeaxanthin, was affected significantly by the irradiance, temperature, and their interaction (Fig. 3).

The range of changes in *β*-carotene content was from about 0.07 to about 0.19 pg filament $unit^{-1}$ (Fig. 3). The values dropped significantly with an increase in the irradiance and increased slightly with an increase in the temperature. However, at temperature between 15 and 20°C, *β*-carotene content went up slightly at the irradiance above 100 µmol(photon) m^{-2} s^{-1}. Minimal values at the high light intensity were about 37% of maximum at the low light intensity. About 66% of total sum of squares was accounted for by irradiance sum of squares and about 24% for temperature (Table 1), whereas the influence of the interaction between investigated factors was statistically insignificant at significance level of 0.05. Myx content changed from about 0.05 to about 0.21 pg filament $unit^{-1}$ (Fig. 3). In contrast, the filament concentration of Myx increased significantly with the increasing light intensity and dropped with the increasing temperature. The values of Myx concentrations at the low light intensity were only 24% of those found at the high light intensity. However, the temperature and the interaction of both investigated factors had a lower effect on Myx content, which was reflected in variance analysis (Table 1). 70% of total sum of squares was accounted for by irradiance sum of squares, while 20% and 10% were accounted for the temperature and the interaction between the irradiance and temperature, respectively.

In *G. amphibium*, total PB, expressed in pg per filament unit, dropped about 4.7-fold with the increasing light intensity (calculated as the mean for the whole range of temperatures investigated) and about 2.9-fold with the increasing temperature (calculated as the mean value for the whole range of the light intensity investigated) (Fig. 4). On the basis of factorial experiments, it was found that minimal concentration of PB [about 0.4 pg $(\text{filament unit})^{-1}$], noted at the range of about

Table 1. Two-way factorial *ANOVA* of some parameters measured in *G. amphibium* growing at different temperatures [°C] and irradiance levels [μmol(photon) m^{-2} s^{-1}]. *ANOVA* – analysis of variance. α – initial slope of photosynthetic curve; Chl – chlorophyll; df – degrees of freedom; DM – dry mass; F – *Fisher*'s F-test statistic; Mss – mean sum of squares; P_{max} – maximum photosynthetic rate; Ss – sum of squares; [a] – significance at p=0.05.

Parameters	Source of variation	Df	Ss	Mss	F
DM [mg]	irradiation	2	0.46	0.23	931.45[a]
	temperature	2	0.59	0.30	1,192.96[a]
	interaction	4	0.28	0.07	272.36[a]
	error	27		0.00	
	total		1.33		
Chl *a* [pg (filament unit)$^{-1}$]	irradiation	2	5.20	2.60	333.34[a]
	temperature	2	0.14	0.07	8.73[1]
	interaction	4	0.07	0.02	2.28[1]
	error	27		0.01	
	total		5.41		
β-carotene [pg (filament unit)$^{-1}$]	irradiation	2	30,248.67	15,124.33	38.83[a]
	temperature	2	11,034.04	5,517.02	14.17[a]
	interaction	4	4,223.35	1,055.84	2.71
	error	27		389.48	
	total		45,506.06		
myxoxanthophyll [pg (filament unit)$^{-1}$]	irradiation	2	0.07	0.03	63.71[a]
	temperature	2	0.02	0.01	15.89[a]
	interaction	4	0.01	0.00	4.73[a]
	error	27		0.00	
	total		0.10		
phycobilins [pg (filament unit)$^{-1}$]	irradiation	2	57.43	28.71	241.37[a]
	temperature	2	12.98	6.49	54.57[a]
	interaction	4	0.53	0.13	1.11
	error	27		0.12	
	total		70.94		
phycobilins/Chl *a*	irradiation	2	0.74	0.37	3.73[a]
	temperature	2	30.73	15.37	154.63[a]
	interaction	4	3.06	0.76	7.70[a]
	error	27		0.10	
	total		34.54		
DM-specific P_{max} [10^{-2} μl(O_2) mg (DM)$^{-1}$ h^{-1}]	irradiation	2	177.69	88.84	336.57[a]
	temperature	2	277.37	138.68	525.38[a]
	interaction	4	145.23	36.31	137.54[a]
	error	18		0.26	
	total		600.29		
Chl *a*-specific P_{max} [10^{-2} μl(O_2) μg (Chl *a*)$^{-1}$ h^{-1}]	irradiation	2	300.39	150.19	1,281.14[a]
	temperature	2	160.02	80.01	682.48[a]
	interaction	4	81.24	20.31	173.25[a]
	error	18		0.12	
	total		541.65		
DM-specific α [10^{-2} μl(O_2) mg (DM)$^{-1}$ h^{-1} (μmol(photon) m^{-2} s^{-1})$^{-1}$]	irradiation	2	0.58	0.29	532.08[a]
	temperature	2	0.12	0.06	110.88[a]
	interaction	4	0.05	0.01	21.19[a]
	error	18		0.00	
	total		0.75		
Chl *a*-specific α [10^{-2} μl(O_2) μg (Chl *a*)$^{-1}$ h^{-1} (μmol(photon) m^{-2} s^{-1})$^{-1}$]	irradiation	2	4,935.31	2,467.65	18.71[a]
	temperature	2	26,4837.31	13,2418.66	1,004.03[a]
	interaction	4	29,507.88	7,376.97	55.93[a]
	error	18		131.89	
	total		299,280.50		

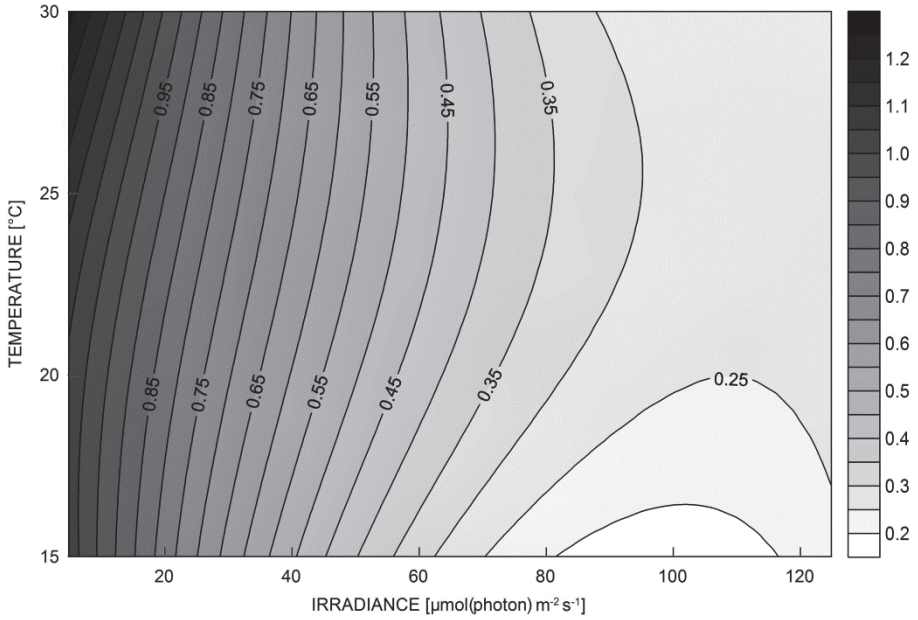

Fig. 2. Response-surface estimation of chlorophyll *a* content in *G. amphibium* expressed in pg (filament unit)$^{-1}$ at 14th d of cultivation at different temperature and irradiance levels.

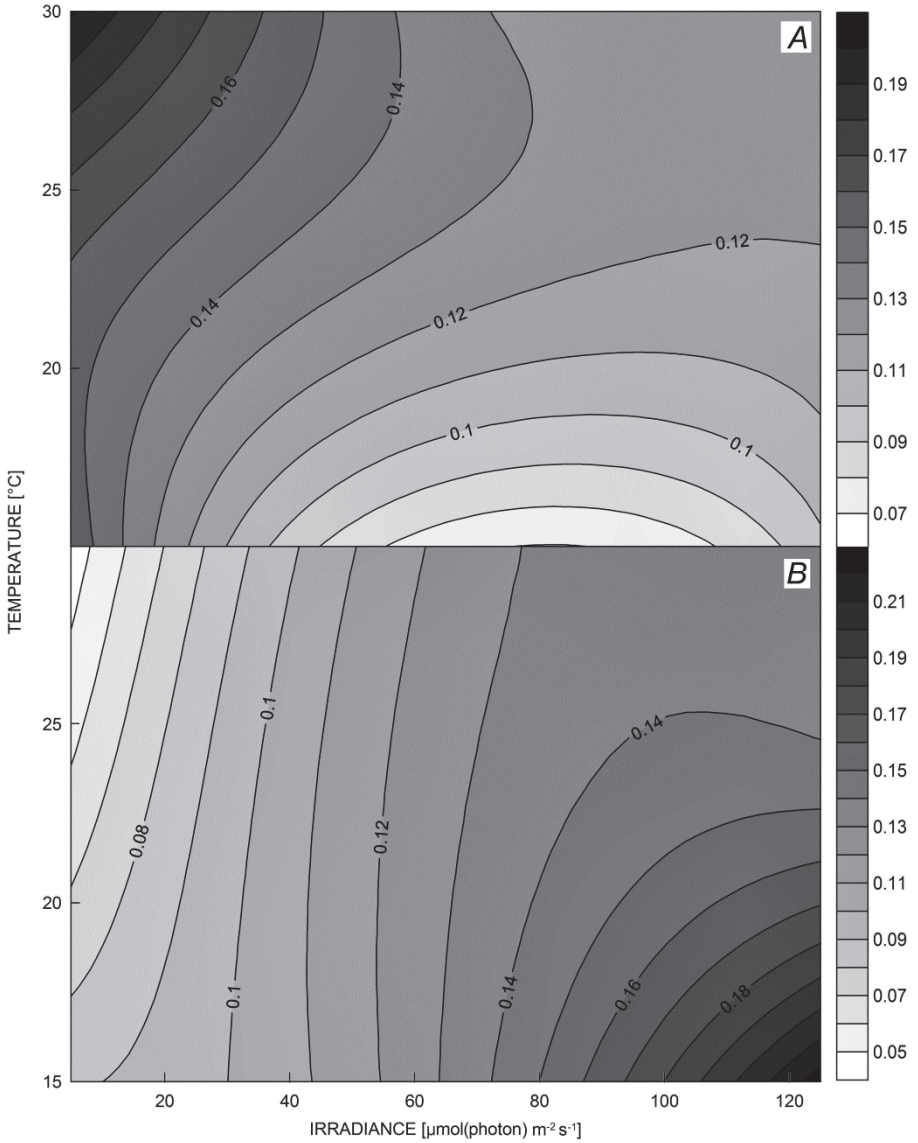

Fig. 3. Response-surface estimation of: (*A*) *β*-carotene and (*B*) myxoxanthophyll content in *G. amphibium* expressed in pg (filament unit)$^{-1}$ at 14th d of cultivation at different temperatures and irradiance levels.

90–125 μmol(photon) m^{-2} s^{-1} and temperature of 28–30°C, was only about 10% of maximal values (about 4.4 pg filament $unit^{-1}$), occurring at the low light intensity of 5–10 μmol(photon) m^{-2} s^{-1} and temperature of 15–18°C. The results of variance analysis showed that 81% and 18.3% of total sum of squares were accounted for by the irradiance and temperature, respectively, whereas the influence of the interaction between the factors investigated was not statistically significant at level of 0.05 (Table 1).

However, the influence of the temperature on PB/Chl *a* ratio was considerably higher than the effect of the irradiance and the interaction of the factors investigated (Table 1). 89% of total sum of squares was accounted for by temperature sum of squares, while merely 2.1% and 8.9% were accounted for the irradiance and the interaction of both investigated factors, respectively.

PI curves and photosynthetic parameters: Photosynthetic rate per DM unit [10^{-2} μl(O_2) mg(DM)$^{-1}$ h^{-1}], P_{max} , and α parameters were always higher at 5 μmol (photon) m^{-2} s^{-1} (low-light treatment) than at 125 μmol(photon) m^{-2} s^{-1} (high-light treatment). To illustrate the course of PI curves, results recorded at 22.5°C were chosen (Fig. 5). The differences between the maximum and minimum for DM-specific P_{max} and α were 2.8-fold and 8.3-fold, respectively. By contrast, P_C and E_K values were higher in the high light than in the low-light treatment. The differences between the maximum and the minimum of P_C and E_K values were 15-fold and 10-fold, respectively. At each temperature, R_D was also higher in the high light than in the low-light-acclimated cyanobacterium, and for the cultures grown at 22.5°C the difference was 1.8-fold.

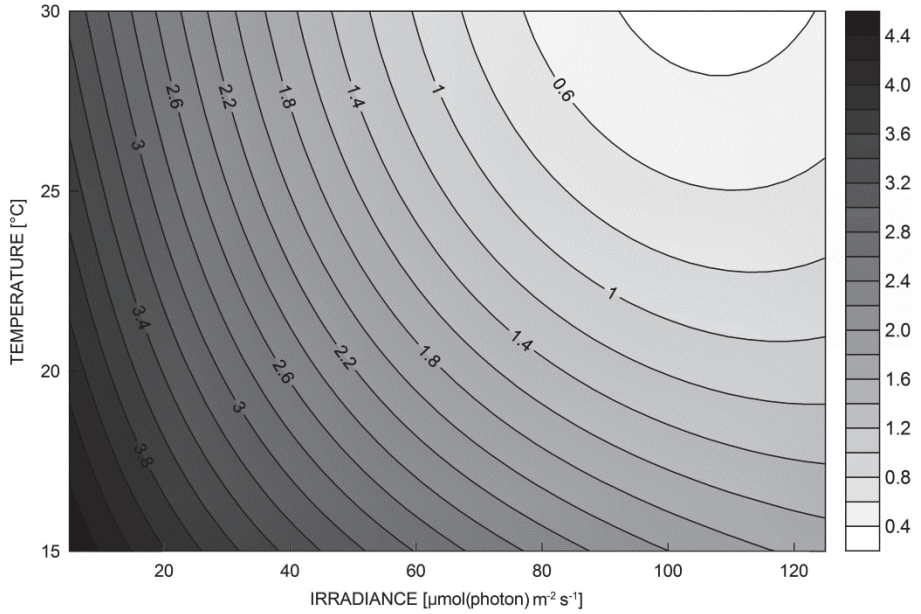

Fig. 4. Response-surface estimation of phycobilin content in *G. amphibium* expressed in pg (filament $unit)^{-1}$ at 14^{th} d of cultivation at different temperatures and irradiance levels.

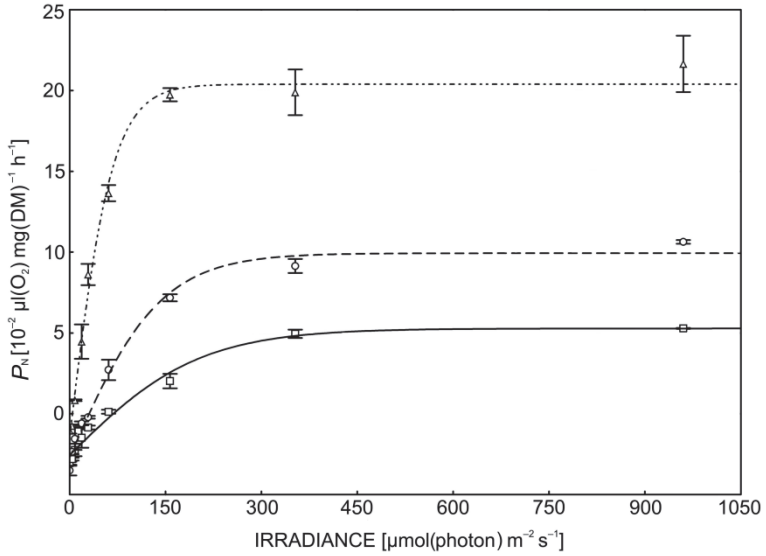

Fig. 5. Net photosynthetic rate (P_N) in *G. amphibium* grown at different irradiance levels [Δ 5, ○ 65, □ 125 μmol(photon) m^{-2} s^{-1}] and temperature of 22.5°C. Mean ± SE (n = 4). DM – dry mass.

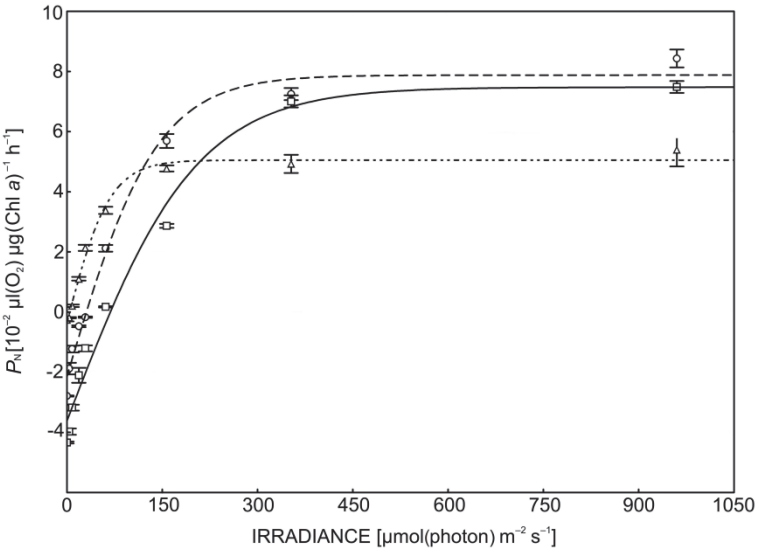

Fig. 6. Net photosynthetic rate (P_N) in *G. amphibium* grown at different irradiance levels [Δ 5, ○ 65, □ 125 µmol(photon) m^{-2} s^{-1}] and temperature of 22.5°C. Mean ± SE (n = 4). Chl – chlorophyll.

When the rate of photosynthesis was expressed per Chl *a* unit [10^{-2} µl(O_2) µg(Chl *a*)$^{-1}$ h^{-1}] (Fig. 6), the values of P_{max}, R_D, and α were different in comparison to those expressed per DM unit, whereas E_K and P_C remained unchanged. Chl *a*-specific P_{max} and R_D were higher in the high-light than in the low-light-acclimated cyanobacterium, and at 22.5°C differed about 2-fold and 10.3-fold for P_{max} and R_D, respectively. In contrast, Chl *a*-specific α parameter remained constant at different growth irradiances.

The use of factorial experimental method and variance analysis made possible to determine the influence of investigated factors and their interaction on photosynthetic parameters and present the results graphically (Fig. 7).

Maximal values of DM-specific P_{max} [about 0.20 µl(O_2) mg(DM)$^{-1}$ h^{-1}] were recorded in the range of the light intensity of 5–20 µmol(photon) m^{-2} s^{-1} and temperature of 20–25°C (Fig. 7). In *G. amphibium*, DM-specific P_{max} was influenced by the irradiance as well as the temperature, but positive effect was connected with the temperature. 46% of total sum of squares was accounted for by temperature sum of squares, while 30% and 24% were accounted for the irradiance and the interaction of investigated factors, respectively (Table 1). On the contrary, maximum of Chl *a*-specific P_{max} (about 0.14 µl(O_2) µg (Chl *a*)$^{-1}$ h^{-1}) was noted at 80–120 µmol(photon) m^{-2} s^{-1} and 15–20°C (Fig. 7). Chl *a*-specific P_{max}, in turn, was more influenced by the irradiance than by the temperature and the interaction of investigated factors. 55% of total sum of squares was accounted for by irradiance sum of squares, whereas 30% and 15% were accounted for temperature and the interaction of investigated factors, respectively (Table 1).

Maximal values of DM-specific α parameter [about 0.55 10^{-2} µl(O_2) mg(DM)$^{-1}$ h^{-1} (µmol(photon) m^{-2} s^{-1})$^{-1}$] were noticed at the low light intensities of 5–20 µmol(photon) m^{-2} s^{-1} and low temperatures of about 15–17°C (Fig. 7). The influence of the irradiance on DM-specific α parameter was considerably higher than the influence of the temperature and the interaction between investigated factors (Table 1). Above 77% of total sum of squares was accounted for by irradiance sum of squares. 16% and 7% were accounted for the temperature and the interaction of investigated factors, respectively. Conversely, Chl *a*-specific α depended strongly on the temperature, while the influence of the irradiance and the interaction of investigated factors was low, but statistically significant at p=0.05. 88.5% of total sum of squares was accounted for by temperature sum of squares, while merely 1.6% and 9.9% were accounted for the irradiance and the interaction of investigated factors, respectively (Fig. 7, Table 1).

Discussion

Cyanobacteria are generally recognized to photosynthesize and grow best at a comparatively lower intensity of light (Fogg and Thake 1987, Ibelings 1996), and as a matter of fact, temperature is the most important parameter for their growth (Davison 1991, Roos and Vincent 1998). In the present study, it was found that the irradiance up to 125 µmol(photon) m^{-2} s^{-1} had stimulating influence on *G. amphibium* biomass production and this effect was additionally increased by the temperature up to 30°C. Based on the experiments with the cyanobacterium *Spirulina platensis*, Jensen and Knutsen (1993) suggested reasons, why higher temperatures, of course, to optimal level, favour better use of high light intensity. An increase in temperature from 20°C to 30°C considerably reduced the photoinhibition, caused by the denaturation of D1 protein. Such positive interaction between minimal

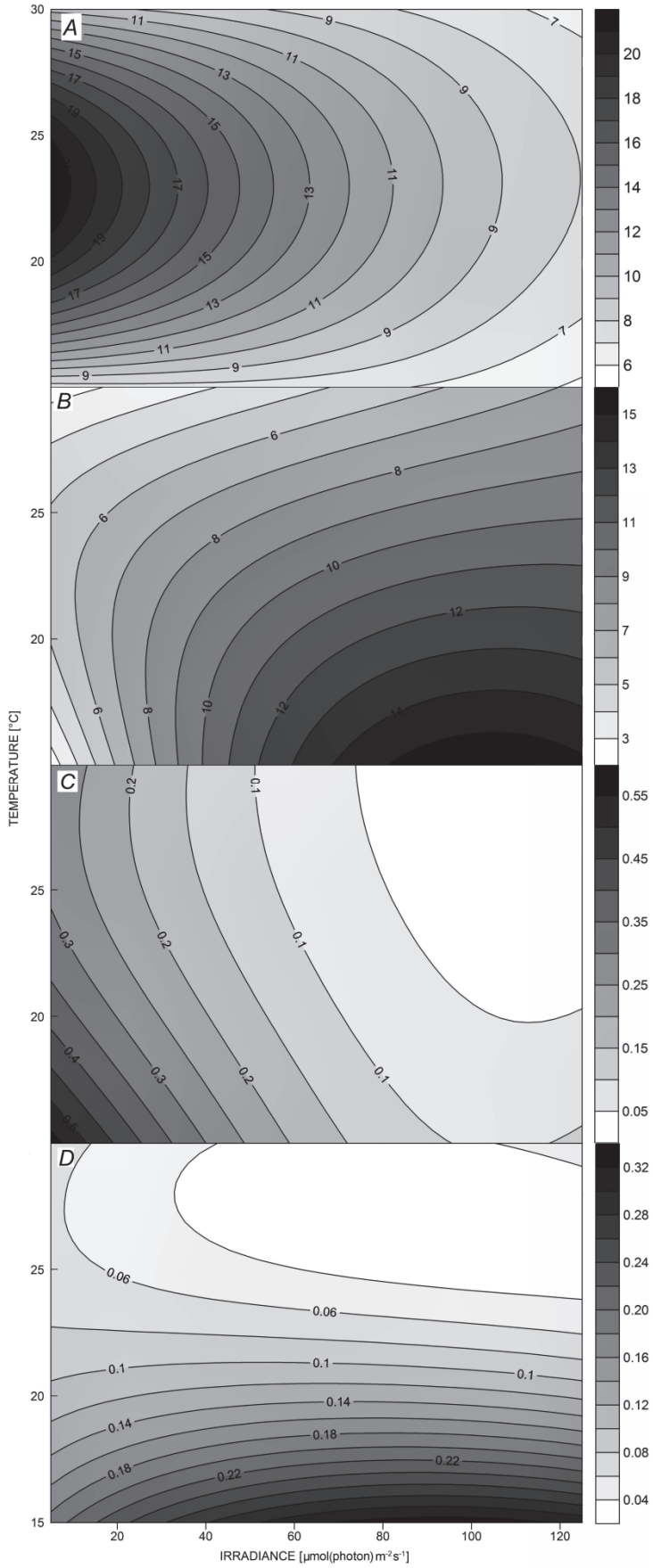

Fig. 7. Response-surface estimation of: (*A*) DM-specific P_{max} [10^{-2} µl(O_2) mg(DM)$^{-1}$ h^{-1}], (*B*) Chl *a*-specific P_{max} [10^{-2} µl(O_2) µg(Chl *a*)$^{-1}$ h^{-1}], (*C*) DM-specific α [10^{-2} µl(O_2) mg(DM)$^{-1}$ h^{-1} (µmol(photon) m^{-2} s^{-1})$^{-1}$], (*D*) Chl *a*-specific α [10^{-2} µl(O_2) µg(Chl *a*)$^{-1}$ h^{-1} (µmol(photon) m^{-2} s^{-1})$^{-1}$] in *G. amphibium* at 14th d of cultivation at different temperatures and irradiance levels. α – initial slope of photosynthetic curve, Chl – chlorophyll, DM – dry mass, P_{max} – maximum photosynthetic rate.

and optimal values of temperature and light intensity for the growth was also reported by Skulberg (1994) in some Oscillatoriales species. In early studies, an increase in *G. amphibium* culture concentration was observed up to 120 μmol(photon) m^{-2} s^{-1} at 35°C as well as up to 170 μmol(photon) m^{-2} s^{-1} at 30°C, whereas the interaction between temperature of 35°C and light intensity of 170 μmol(photon) m^{-2} s^{-1} resulted in the growth inhibition (Latała and Misiewicz 2000). These results suggest that the optimal temperature for the growth of *G. amphibium* is close to 30°C, whereas the optimal light intensity is below 170 μmol(photon) m^{-2} s^{-1}. An excess of light energy absorbed by photosynthetic pigments, together with high-temperature stress, may accelerate the photoinhibition by inhibiting the repair of photodamaged PSII (Allakhverdiev *et al.* 2008, Takahashi and Murata 2008, Takahashi and Badger 2011). However, at lower temperatures of about 15°C, metabolic activity of the organism is on the low level and light intensity does not play important role for the growth of cyanobacterium (Davison 1991, Roos and Vincent 1998).

When *G. amphibium* experienced changes in its light regime, acclimation of photosynthetic apparatus to variable light condition was observed. Photoacclimation of the Baltic cyanobacterium has been linked to alterations in a total cellular concentration of light-harvesting and reaction centre pigments, and also in a ratio of different pigments. In the investigated strain, the changes in Chl *a* content, caused by the irradiance, were relatively high [about 4-fold from 5 to 125 μmol(photon) m^{-2} s^{-1}] in comparison to other cyanobacteria (Raps *et al.* 1983, Kana and Glibert 1987, Millie *et al.* 1990, 1992). The alteration in PB content, triggered by the irradiance change, was similar to the alteration in Chl *a* content, whereas the effect of the temperature on these pigments was opposite. Thus, a ratio of PB/Chl *a* changed slightly under the influence of the irradiance, but it was considerably affected by the temperature. Principal changes in PB content during photoacclimation process come from changes in a length of PB-containing distal rods and the number of phycobilisomes in thylakoids (Tandeau de Marsac 1991, MacIntyre *et al.* 2002). Changes in the number and in the size of phycobilisomes in *G. amphibium* probably run parallel to changes at the antenna number and size of photosynthetic units. In contrast, the PB/Chl *a* ratio significantly dropped with the increasing light intensity in *Synechococcus* strain WH7803 (Kana and Glibert 1987), *Oscillatoria agardhii* (Millie *et al.* 1990), and *Anabaena circinalis* (Millie *et al.* 1992), which means that in above mentioned cyanobacteria, PB content declined more than that of Chl *a*.

The decrease in the content of essential photosynthetic pigments (Chl *a*, PB) in *G. amphibium* filament was concomitant with an accumulation of Myx, which content was as much as 6 times higher in the cultures, grown at 125 μmol(photon) m^{-2} s^{-1} than at 5 μmol(photon) m^{-2} s^{-1}. However, *β*-carotene filament concentration slightly increased (about 1.5 times) with the decreasing light intensity. The effect of the irradiance on the *β*-carotene filament content was in accordance with the results of Millie *et al.* (1990), who compared the pigment content in *O. agardhii* within the range of 50–230 μmol(photon) m^{-2} s^{-1}. The drop in the cellular *β*-carotene content was also small and it was 1.4-fold. These findings indicate that Myx is the main photoprotective carotenoid in *G. amphibium*. According to the literature, this carotenoid is the pigment involved in photoprotection (Raps *et al.* 1983, Millie *et al.* 1990, Steiger *et al.* 1999, Lakatos *et al.* 2001), but many researches indicate zeaxanthin as the carotenoid, which plays an important role in photoprotection of the cyanobacterial photosynthetic apparatus (Pearl *et al.* 1983, Kana *et al.* 1988, Rau 1988, Bidigare *et al.* 1989). However, zeaxanthin filament concentration in *G. amphibium* remained constant over the whole range of factors tested (~0.08 pg filament $unit^{-1}$), which could suggest rather minor role of zeaxanthin in photoprotection of *G. amphibium*. Photoprotective carotenoids are capable of quenching the triplet state of photosensitizing molecules, singlet oxygen and free radical intermediates, all of which are potentially destructive (Rau 1988, Takahashi and Murata 2008, Takahashi and Badger 2011). Moreover, in cyanobacteria, a soluble carotenoid-binding protein is associated with thermal energy dissipation and suppressed energy transfer from antenna proteins (phycobilisomes) to the photosystems (Takahashi and Badger 2011).

We hypothesize that *G. amphibium* evolved two mechanisms of the photoacclimation. Cultures acclimated to the low light intensity had considerably higher DM-specific P_{max}, which increase resulted from the increase in a number of reaction centres available. However, if the only strategy of *G. amphibium* acclimation were changes in PSU number, Chl *a*-specific P_{max} would be constant (Dring 1998). Based on our investigation, it was found that Chl *a*-specific P_{max} was significantly lower in *G. amphibium* acclimated to the low light intensity. This suggests that cyanobacterium responded to the low irradiance not only by building up completely new PSU, but also by adding extra pigment molecules to the existing PSU. Another mechanism of the acclimation was connected with a drop in R_D together with a decrease in the light intensity. Fisher *et al.* (1996) suggest that changes in R_D result directly from changes in the growth rate. Moreover, Prezelin and Matlick (1980) found a correlation between increasing R_D and increasing growth rate and a decrease in photosynthetic pigments. In the present study, the same relationship between the culture concentration, R_D, and pigment concentration was also found. Ample data on the relationship of R_D and growth rate, in the context of photoacclimation, are presented by Falkowski *et al.* (1985). The increase in E_K and P_C with increasing growth irradiance is characteristic for photoautotrophic organisms in accordance with the relevant literature (Richardson *et al.* 1983). However, it should be

emphasized that ranges of the change for both photosynthetic parameters were very wide, 20–200 μmol(photon) m^{-2} s^{-1} and 5–75 μmol(photon) m^{-2} s^{-1} for E_K and P_C, respectively. E_K and P_C parameters can inform about light requirements of different photoautotrophic species (Rabinowitch 1951, Latała 1991). Achieved results for both parameters showed a good acclimation capacity of species investigated to irradiance changes. The minimal values of E_K and P_C indicated a shadow-tolerant character of *G. amphibium*, whereas the maximal ones pointed at its heliophylous character. It is also noteworthy that PI curves for *G. amphibium* did not indicate photosynthetic photoinhibition until approximately 1,000 μmol(photon) m^{-2} s^{-1}, even if the cyanobacterium were acclimated to very low light intensity [5 μmol(photon) m^{-2} s^{-1}]. Photoihibition is a function of irradiance and duration of exposure, but it is also affected by the interaction between temperature and nutrition. It also depends on a genotype as well as past and present environmental conditions (Henley 1993). In the review of published data on natural phytoplankton population, photoinhibition often starts to occur at irradiances about 200 μmol(photon) m^{-2} s^{-1} , but it can emerge in many species at lower irradiances (Richardson *et al.* 1983). It might, additionally, prove the exceptional capacity of the investigated cyanobacterium for the acclimation to an excessive light. However, in the present work, no reduction in P_{max} during the exposure even to 1,000 μmol(photon) m^{-2} s^{-1} might result from too short time of exposure, which was only 25 min during the measurement. Ample data on the relationship of photoinhibition and duration of exposure are presented by Henley (1993).

It was also evident that photosynthetic activity of *G. amphibium* was very sensitive to changes in temperature. Both P_{max} and α were found to decline with an increase in the temperature and, in the case of the latter parameter, the negative effect of the temperature was gradually intensified by its interaction with the increasing irradiance. These results were in accordance with the observation of Ibelings (1996), who noticed a decrease in photosynthetic rate and α parameter at temperatures of 30°C and above. However, irradiance of 300 μmol(photon) m^{-2} s^{-1}, but especially 1,000 μmol(photon) m^{-2} s^{-1}, intensified the damaging effects of the temperature. According to Wilhelm (1993), changes in P_{max} are often determined by changes in activity of Rubisco or the rate of linear electron transport, which are the most temperature-sensitive. A decrease in photosynthetic activity of *G. amphibium* at higher temperatures (above 25°C) might be partially connected with a decrease in light absorption by PB; their ratio to Chl *a* considerably declined at high temperatures. Moreover, the observed increase in Myx/Chl *a* and zeaxanthin/Chl *a* ratios at low temperature might reflect an increasing need for photoprotection by carotenoids and it could suggest a good acclimation of the investigated cyanobacterium to lower temperatures (Davison 1991). Similar effects of a low temperature on carotenoids/Chl *a* ratio were observed in the Antarctic mat-forming cyanobacterium, *Phormidium murrayi* (Roos and Vincent 1998). At high light intensity [500 μmol(photon) m^{-2} s^{-1}], the ratio at 10°C was even 2 times higher as compared with 20°C. In contrast, the investigation of Tang *et al.* (1997), on 24 species of *Phormidium* and 3 species of *Oscillatoria*, showed that two thirds of them responded to low temperature by increasing carotenoids/Chl *a* ratio, and between temperatures of 35°C and 5°C, the increase was on the average 5-fold.

In conclusion, a wide range of changes in the growth, pigment composition, as well as photosynthetic activity of *G. amphibium* indicated a good acclimation ability to dynamically changing environmental condition. The capacity of acclimation in *G. amphibium* was accomplished in part by adjustments in photosynthetic pigments, their content, and relative proportions, which in turn reflected characteristic pattern in the course of photosynthetic light response curves.

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