DOI: 10.1007/s11099-014-0024-y PHOTOSYNTHETICA 52 (2): 223-232, 2014

Effects of light intensity and temperature on the photosynthetic irradiance response curves and chlorophyll fluorescence in three picocyanobacterial strains of *Synechococcus*

S. JODŁOWSKA+ and S. ŚLIWIŃSKA

Laboratory of Marine Plant Ecophysiology, Institute of Oceanography, University of Gdańsk, Piłsudskiego Av. 46, 81-378 Gdynia, Poland

Abstract

Chrococcoid cyanobacteria of the genus *Synechococcus* are the important component of marine and freshwater ecosystems. Picocyanobacteria comprise even 80% of total cyanobacterial biomass and contribute to 50% of total primary cyanobacterial bloom production. Chlorophyll (Chl) fluorescence and photosynthetic light response (*P-I*) curves are commonly used to characterize photoacclimation of *Synechococcus* strains. Three brackish, picocyanobacterial strains of *Synechococcus* (BA-132, BA-124, BA-120) were studied. They were grown under 4 irradiances [10, 55, 100, and 145 μmol(photon) m^{-2} s^{-1}] and at 3 temperatures (15, 22.5, and 30°C). Photosynthetic rate was measured by Clark oxygen electrode, whereas the Chl fluorescence was measured using Pulse Amplitude Modulation fluorometer. Based on *P-I*, two mechanisms of photoacclimation were recognized in *Synechococcus*. The maximum value of maximum rate of photosynthesis (P_{max}) expressed per biomass unit at 10 μmol(photon) m^{-2} s^{-1} indicated a change in the number of photosynthetic units (PSU). The constant values of initial slope of photosynthetic light response curve (α) and the maximum value of P_{max} expressed per Chl unit at 145 μmol(photon) m^{-2} s^{-1} indicated another mechanism, *i.e.* a change in PSU size. These two mechanisms caused changes in photosynthetic rate and its parameters (compensation point, α, saturation irradiance, dark respiration, P_{max}) upon the influence of different irradiance and temperature. High irradiance had a negative effect on fluorescence parameters, such as the maximum quantum yield and effective quantum yield of PSII photochemistry (Φ_{PSII}), but it was higher in case of Φ_{PSII}.

Additional key words: fluorescence; irradiance; photoacclimation; photosynthesis; picocyanobacteria; temperature

Introduction

Late-summer blooms of cyanobacteria are common in Baltic Sea, the largest brackish water basin in the world. Toxic blooms cause serious sanitary and epidemiological problems (Blackburn *et al.* 1996, Kahru *et al.* 2000, Panosso and Graneli 2000). However, even nontoxic species can disturb significantly ecological balance. Dead organic matter can lead to oxygen shortage, and in turn to a production of toxic hydrogen sulphide. Low-quality water, characterized by an unpleasant smell and debris, affects negatively recreation and tourism and it causes economic losses. Nevertheless, cyanobacteria are permanent components of water ecosystem and they are essential primary producers in an environmental food chain.

Chrococcoid, cyanobacteria strain of the genus *Synechococcus* appears both in marine and freshwater ecosystems. In Baltic Sea, cyanobacterial blooms are comprised of two morphologically different fractions: larger, filamentous fraction (mainly *Nodularia spumigena*, *Aphanizomenon flos-aquae*, and *Anabaena* sp.) and small, unicellular picocyanobacteria (*Synechococcus* sp.) (Stal *et al.* 2003, Hajdu *et al.* 2007). Despite their small cell size (majority less than 1 μm), picocyanobacteria are able to

Received 27 February 2013, *accepted* 2 September 2013.
+Corresponding author; phone: +48 58-5236892, fax: +48 58-5236678, e-mail: ocesm@univ.gda.pl
Abbreviations: *ANOVA* – analysis of variance; CCBA – Culture Collection of Baltic Algae; Chl – chlorophyll; I_K– saturation irradiance; F_V/F_M – maximum quantum yield of PSII photochemistry; N – number of cells; OD – optical density; PAR – photosynthetically active radiation; PAM – Pulse Amplitude Modulation; CP – compensation point; *P-I* – photosynthetic light response; P_{max} – maximum photosynthetic rate; P_N – net photosynthetic rate; PSU – photosynthetic units; R_D – dark respiration rate; TEM – transmission electron microscope; Φ_{PSII} – effective quantum yield of PSII photochemistry; α – initial slope of photosynthetic light response curve.
Acknowledgements: This study was supported by research grant issued by the Council for Science - Poland (N304 3278 36) and by the University of Gdańsk (BW/G245-5-0233-9, BW/G245-5-0502-0).

comprise even 80% of total cyanobacterial biomass and contribute to 50% of total primary cyanobacterial bloom production (Stal and Walsby 2000, Stal *et al.* 2003). Stal *et al.* (2003) estimated that filamentous cyanobacteria contributed on average up 13% of the phytoplankton community, whereas picocyanobacteria (*Synechococcus* sp.) about 21% of total phytoplankton Chl *a*.

Many adaptation strategies developed by picoplanktonic organisms allowed them to spread in aquatic environment and dominate consequently the niche inaccessible for other photoautotrophs. Owing to the fact that picocyanobacteria exhibit the small size of cells and possess an advantageous surface area to volume ratio, they can assimilate trace amounts of nutrients**.** Therefore, in oligotrophic regions of seas and oceans, picoplankton can compete successfully with larger algae and determine primary production of the whole water ecosystem (Six *et al.* 2007, Richardson and Jackson 2007). However, the increase of nutrients available can decrease the advantage of picoplankton over larger algae cells causing significantly drop in their biomass and primary production share. Picocyanobacteria can effectively float in the ocean water due to their small size and despite the lack of gas vesicles.

Growth intensity and distribution of picocyanobacteria are determined by their optimal ecological requirements, such as light, temperature, and availability of nutrients. These factors influence metabolic processes, photosynthetic activity, and consequently the rate of cell division and growth.

In earlier studies, Jodłowska *et al.* (2010) described the effect of light intensity on the growth and pigment composition of three different, brackish, picocyanobacterial strains from the genus *Synechococcus.* This encouraged us to recognize and describe cyanobacterial photoacclimation mechanisms. The main aim was to estimate the effect of irradiance and temperature on *P-I* curves and Chl fluorescence. Studies of the cyanobacterial species autecology and recognition of their reactions to the main environmental factors might contribute to further research of the cyanobacterial blooms in the aquatic ecosystems.

Materials and methods

Material and experimental conditions: Three different phenotypes of picocyanobacterial strains were examined from the genus *Synechococcus*: BA-120 (red), BA-132 (yellow), and BA-124 (green). The strains were isolated in late spring of 2002 from coastal zone of the Gulf of Gdańsk (southern Baltic Sea) and they have been maintained as unialgal cultures in Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, University of Gdańsk, Poland (Latała *et al.* 2006).

Batch cultures were grown in sterilized BG-11 medium (Stanier *et al.* 1971) in 100 ml glass Erlenmeyer flasks. Media were prepared from Baltic water of salinity 8, previously filtered through *Whatman GF/C* filters. Strains were incubated under a 16/8 h of light/dark cycle at 4 irradiances [10, 55, 100, and 145 μmol(photon) m^{-2} s^{-1}]. Fluorescent lamps (*Cool White 40W, Sylvania*, USA) were used as source of irradiance and combined with halogen lamps (*100W, Sylvania*, USA) to obtain more intensive light. The intensity of PAR was measured using a quantum-meter (*LI-189, LI-COR Inc.*, Nebraska, USA) with a cosine collector. Experiments were conducted in small incubators capable of maintaining constant temperature condition (± 1°C) at 3 temperatures (15, 22.5, and 30ºC). Specimens were acclimated to each condition for 7 d. Then they served as inoculum for test cultures, where the initial number of cells was 10^7 per ml. Test cultures were grown in 3 replicas and were incubated for 2 weeks at each combination of light and temperature. On the last day of incubation in exponential growth phase, Chl *a* fluorescence and *P-I* were measured in each replica.

Culture concentration was determined using the number of cells (N) and optical density (OD). N was estimated in *Bürker* chamber with light microscope (*Nikon Eclipse 80i*, *Nikon*, Tokyo, Japan). OD was measured spectrophotometrically at 750 nm with *DU530 UV-VIS Life Science* spectrophotometer (*Beckman*, USA) in a 1-cm glass cuvette. The data provided the basis for determining the value of the correlation coefficient (r = 0.987, 0.992, and 0.993, for BA-120, BA-124, and BA-132, respectively) and linear correlation between N and OD (y [N ml^{-1}] = $88.11\ 10^7\ x - 69.9\ 10^3$; y [N ml^{-1}] = $92.72\ 10^6\ x - 4.0\ 10^5$, and y [N ml^{-1}] = $46.28\ 10^7\ x - 59.7\ 10^4$, for BA-120, BA-124, and BA-132, respectively, where y = N per ml, and x = OD). Cell concentrations in test cultures was determined optically in each culture and then derived from the calibration curve mentioned above.

Chl concentration: Chl *a* was extracted in 90% acetone at –20ºC for 2 h in darkness. After centrifugation at 2,124 × *g* for 5–10 min, the absorbance of pigment extract was measured at 665 and 750 nm with a *UV-VIS* spectrophotometer (*DU 530, Beckman Coulter Inc.*, Pasadena, California, USA). The concentration of Chl *a* was calculated by the formula: Chl *a* [μg ml^{-1}] = $11.236\ (E_{665} - E_{750})\ V_a/V_b$, derived on the basis of a factor by Strickland and Parsons (1972), 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.

Chl fluorescence was measured using PAM method by fluorometer (*FMS1, Hansatech*, King's Lynn, Norfolk, UK) (Maxwell and Johnson 2000). All light sources required for the modulated measurement of fluorescence parameters were supplied by 594-nm amber modulating beam, dual-purpose halogen actinic/saturating pulse lamp,

and 735-nm far-red LED sources. Chl fluorescence measurements were determined after about 30 min of dark adaptation (Jodłowska and Latała 2010). F_V/F_M reflects the maximum photochemical efficiency of PSII in the dark-adapted state, whereas Φ_{PSII} represents the actual photochemical efficiency of PSII under actinic light intensity.

Gas-exchange rate: Measurements of photosynthesis and dark respiration rate were carried out using Clark oxygen electrode (*Chlorolab 2, Hansatech,* King's Lynn, Norfolk, UK). Temperature was controlled by cooling system *LAUDA* (*E100*, Germany). Irradiance was measured with quantum sensor (*Quantitherm, Hansatech*, King's Lynn, Norfolk, UK). To estimate *P-I* curve, samples were always taken from the dark phase of light/dark cycle. Dark respiration rate (R_D) was determined at first. Oxygen production was measured within a range 0–900 μmol (photon) m^{-2} s^{-1}. The course of *P-I* curves was fitted to the data using the *STATISTICA®6.0* software program and mathematical function of Platt and Jassby (1976). The following photosynthetic parameters were determined: compensation point (CP), saturation irradiance (I_K), α, P_{max}, and R_D.

Electron microscopic studies: Morphological observations of thylakoid ultrastructure and cell size were performed when cyanobacteria were grown 14 d under two extreme light intensities: 10 and 145 μmol(photon) m^{-2} s^{-1}. Measurements were done on at least 100 cells. Samples for transmission electron microscopy (TEM) were prepared and embedded following previously described procedure (Surosz and Palińska 2000). Cells were fixed in 2–5% glutaraldehyde in cacodylate buffer of pH 7.4 at 4ºC for 1 h and postfixed in 1% osmic acid for 2 h. The tissues were then dehydrated through graded ethanol and propylene oxide mixtures and embedded in *Epon 812*. All micrographs were taken at 15,500 × magnification (*Philips CM100*, Adelaide, Australia).

Factorial experiments and statistical analysis: The effect of PAR, temperature, and their interaction on photosynthetic activity of examined strains were compared by factorial experiments and two-way analysis of variance (*ANOVA)* at a confidence level of $p<0.05$. Statistical calculations were performed using the *STATISTICA® 6.0* program.Values of independent variables occurred at the same intervals and a replicate number was always the same. All experimental variants run in triplicate. It enabled calculations by creating polynomials that were mutually orthogonal. The fitting of the polynomial by the method ξ' (ksi prim) (Snedecor and Cochran 1980, Oktaba 1986) was simplified by the use of tables of orthogonal polynomials (Fisher and Yates 1963). This method made possible to determine the influence of factors investigated and their interaction on measured parameters by calculation of regression equation. *STATISTICA® 6.0* was used to find best regression equation and results were presented graphically as response surface form using *Golden Software Surfer 8.0*.

Results

Microscopic observation: *Synechococcus* cells cultured under different light intensities varied in the number of thylakoid membranes. The mean values of thylakoid membrane number (n = 100) for *Synechococcus* strains were shown in Table 1. Cyanobacteria growing at 10 μmol (photon) m^{-2} s^{-1} had average values equal to 2.63, 2.73, and 2.77, whereas at 145 μmol(photon) m^{-2} s^{-1} the same values were 1.38, 1.43, and 1.47, respectively for BA-120, BA-124 and BA-132. Selected ultrastructures of thylakoid membranes were presented on Fig. 1. Light intensity had no influence on cell size. *Synechococcus* BA-120 cell size ranged from 1.33 to 2.26 μm (with the mean of 1.65 μm), while BA-124 varied from 1.39 to 2.43 μm (with the mean of 1.75 μm), and BA-132 from 1.59 to 2.88 μm (with the mean of 2.04 μm).

P-I: When parameters were expressed per cell unit (cell-specific, [fmol(O_2) $cell^{-1}$ h^{-1}]), P_{max} and α were always the highest at 10 μmol(photon) m^{-2} s^{-1} and the lowest at 145 μmol(photons) m^{-2} s^{-1}. To illustrate the course of *P-I* curves, results recorded at 22.5°C were chosen (Fig. 2*A–C*, Table 2). Maximum values of cell-specific P_{max} were 46%, 22%, and 59% higher than the minimum values in BA-120, BA-124, and BA-132, respectively. However, maximum values of cell-specific α parameter were 67, 85, and 56% higher than the minimum for BA-120, BA-124, and BA-132, respectively. By contrast, CP and I_K values were the highest under the highest light treatment and the lowest at the lowest light treatment. Maximum values of CP were 77%, 92%, and 81% higher than the minimum for BA-120, BA-124, and BA-132, respectively. The differences between maximal and minimal values of I_K were 60%, 81%, and 27% in BA-120, BA-124, and BA-132, respectively.

When parameters were calculated per unit of Chl *a* (Chl *a*-specific, [μmol(O_2) mg(Chl *a*) h^{-1}]) (Fig. 2*D–F*, Table 3), the values of P_{max}, R_D, and α were different in comparison to those expressed per cell unit, whereas I_K and CP remained unchanged. Chl *a*-specific P_{max} were the highest under

Table 1.The mean values of thylakoid membrane number ± SD (n = 100) in *Synechococcus* strains BA-120, BA-124, and BA-132 after 14 d of cultivation at 2 irradiances and 22.5°C.

Growth irradiance [μmol(photon) m^{-2} s^{-1}]	Thylakoid membrane number		
	BA-120	BA-124	BA-132
0	2.63 ± 0.22	2.73 ± 0.25	2.77 ± 0.25
145	1.38 ± 0.22	1.43 ± 0.18	1.47 ± 0.12

Table 2. Parameters of *P-I* curves [per cell unit] in *Synechococcus* strains BA-120, BA-124, and BA-132 after 14 d of cultivation at 4 irradiance levels and 22.5°C. *P-I* – photosynthetic light response. Values are the means ± SD (n = 3) derived from the *P-I* curves. P_{max} and R_D [fmol(O_2) cell^{-1} h^{-1}]; P_C and I_K μmol(photon) m^{-2} s^{-1}]; α [fmol(O_2) cell^{-1} h^{-1}/μmol(photon) m^{-2} s^{-1}]. α – initial slope of *P-I* curve; I_K – saturation irradiance; P_C – compensation point; P_{max} – maximum photosynthetic rate; R_D – dark respiration rate.

Growth irradiance [μmol(photon) m^{-2} s^{-1}]	Strain	Gross P_{max}	R_D	P_C	I_K	α
10	BA-120	2.1 ± 0.2	-0.3 ± 0.2	10.3 ± 0.8	115.7 ± 14.6	0.012 ± 0.003
	BA-124	1.5 ± 0.0	-0.2 ± 0.1	4.9 ± 1.5	35.8 ± 3.1	0.041 ± 0.004
	BA-132	3.5 ± 0.0	-0.4 ± 0.1	12.7 ± 4.7	183.3 ± 7.6	0.018 ± 0.001
55	BA-120	2.1 ± 0.0	-0.4 ± 0.0	22.7 ± 0.5	152.5 ± 2.7	0.012 ± 0.000
	BA-124	1.2 ± 0.0	-0.1 ± 0.0	9.4 ± 0.6	100.4 ± 4.2	0.012 ± 0.001
	BA-132	1.8 ± 0.3	-0.3 ± 0.1	40.5 ± 1.9	187.6 ± 64.5	0.012 ± 0.005
100	BA-120	1.7 ± 0.1	-0.3 ± 0.1	29.0 ± 5.7	201.5 ± 2.7	0.008 ± 0.001
	BA-124	1.3 ± 0.1	-0.4 ± 0.0	35.3 ± 3.4	129.2 ± 12.5	0.010 ± 0.001
	BA-132	1.9 ± 0.0	-0.6 ± 0.1	57.7 ± 3.9	207.7 ± 5.6	0.009 ± 0.001
145	BA-120	1.1 ± 0.0	-0.2 ± 0.0	43.9 ± 5.9	291.5 ± 33.4	0.004 ± 0.001
	BA-124	1.2 ± 0.0	-0.4 ± 0.0	58.4 ± 0.4	180.6 ± 7.8	0.006 ± 0.000
	BA-132	1.4 ± 0.0	-0.4 ± 0.1	66.8 ± 1.1	251.1 ± 11.9	0.008 ± 0.001

Table 3. Parameters of *P-I* curves [per Chl *a* unit] in *Synechococcus* strains BA-120, BA-124, and BA-132 after 14 d of cultivation at 4 irradiance levels and 22.5°C. Chl – chlorophyll; *P-I* – photosynthetic light response. Values are the means ± SD (n = 3) derived from the *P-I* curves. α – initial slope of *P-I* curve [μmol(O_2) mg(Chl *a*) h^{-1}/μmol(photon) m^{-2} s^{-1}; P_{max} – maximum photosynthetic rate [μmol(O_2) mg(Chl *a*) h^{-1}]; R_D – dark respiration rate [μmol(O_2) mg(Chl *a*) h^{-1}].

Growth irradiance [μmol(photon) m^{-2} s^{-1}]	Strain	Gross P_{max}	R_D	α
10	BA-120	396.6 ± 41.6	–116.0 ± 31.4	5.1 ± 1.0
	BA-124	539.8 ± 9.3	–61.3 ± 15.7	8.4 ± 0.8
	BA-132	469.9 ± 26.9	–61.9 ± 30.5	3.1 ± 1.1
55	BA-120	882.3 ± 17.0	–202.8 ± 1.3	8.9 ± 0.2
	BA-124	725.5 ± 3.6	–74.0 ± 7.8	6.8 ± 0.3
	BA-132	551.9 ± 38.9	–111.4 ± 54.7	3.8 ± 0.4
100	BA-120	1,181.6 ± 15.3	–210.5 ± 10.7	6.7 ± 0.6
	BA-124	961.8 ± 64.6	–166.8 ± 5.8	7.2 ± 0.2
	BA-132	924.6 ± 5.5	–204.1 ± 19.8	3.7 ± 0.4
145	BA-120	1,547.2 ± 114.7	–205.2 ± 11.	5.4 ± 0.7
	BA-124	1,335.3 ± 17.1	–237.8 ± 23.6	6.6 ± 0.6
	BA-132	1,157.2 ± 9.3	–145.0 ± 6.8	4.1 ± 0.1

the highest light treatment. The maximum values of Chl *a*-specific P_{max} at 22.5°C were 74%, 60%, and 59% higher than the minimum ones in BA-120, BA-124, and BA-132, respectively. In contrast, differences in Chl *a*-specific α parameter caused by irradiance were not statistically significant.

Factorial experimental method and *ANOVA* enabled to determine the influence of investigated factors and their interaction on photosynthetic parameters. The results were presented graphically in response surface form (Fig. 3).

Values of cell-specific P_{max} were the highest in the strains grown under the low light intensity [10–60 μmol (photon) m^{-2} s^{-1}] and the high temperature (28–30°C). They displayed the decrease with increasing growth irradiance and decreasing temperature (Fig. 3*A-C*). Maximum values were about 74%, 53%, and 82% higher than the minimum for BA-120, BA-124, and BA-132, respectively. However, in *Synechococcus* BA-132, changes caused by irradiance and temperature of cell-specific P_{max} were not statistically significant below 22.5°C.

Cell-specific α parameter was influenced by light intensity and temperature, with the stronger effect caused by irradiance, which was evident only in BA-120 and BA-124 (Fig. 3*D–F*). Maximum values of cell-specific α noted under the intensity of about 10–20 μmol(photon) m^{-2} s^{-1} were about 89% and 86% higher in BA-120 and BA-124, respectively, than the minimum ones under light intensity of 120–145 μmol(photon) m^{-2} s^{-1}. In BA-132, statistically significant influence of light intensity was observed only under 20–25 μmol(photon) m^{-2} s^{-1}, however, the effect was not statistically significant outside this examined range.

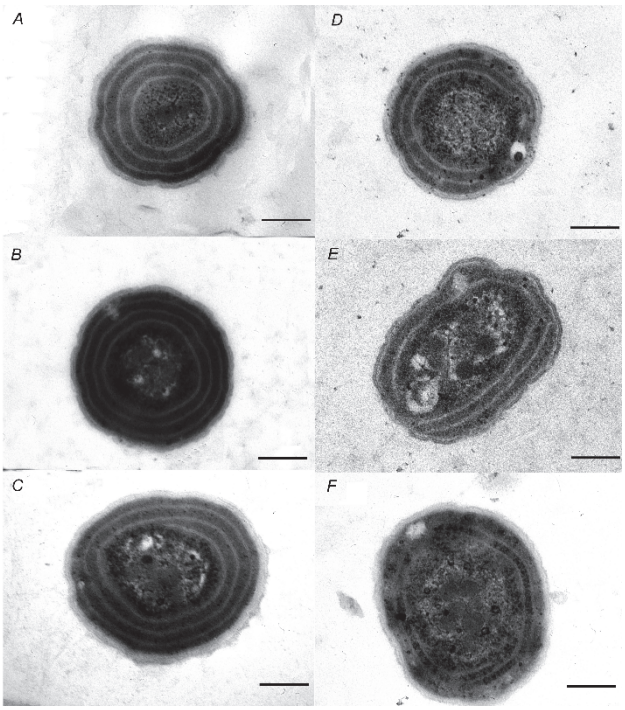

Fig. 1. Ultrastructure of thylakoids in *Synechococcus* strains: (*A*) BA-120, (*B*) BA-124, (*C*) BA-132 growing at 10 μmol(photon) m^{-2} s^{-1}, and (*D*) BA-120, (*E*) BA-124, (*F*) BA-132 growing at 145 μmol(photon) m^{-2} s^{-1}. All micrographs are taken at 15,500 × magnification. Scale bar = 0.5 μm.

Chl fluorescence: Effects of growth irradiance and temperature on Chl fluorescence were shown in Fig. 4. Results of *ANOVA* showed the influence of irradiance on F_V/F_M and Φ_{PSII} was higher than the influence of temperature and the interaction of both factors. However, in BA-120, the stronger effect of temperature was noted in F_V/F_M. In general, both F_V/F_M and Φ_{PSII} were affected negatively by irradiance and positively by temperature in all strains. Maximum values of both parameters were found after the low-light and high-temperature treatment in all strains of *Synechococcus*. The maximum values of F_V/F_M were about 82%, 69%, and 91% higher than minimum for BA-120, BA-124, and BA-132, respectively. The maximum values of Φ_{PSII} were about 83%, 83%, and 85% higher than minimum for BA-120, BA-124, and BA-132, respectively. In every case, the values obtained for Φ_{PSII} were higher than for F_V/F_M, and maximum values of Φ_{PSII} were about 35%, 27% and 21% higher than maximum values of F_V/F_M for BA-120, BA-124, and BA-132, respectively.

Discussion

Most picocyanobacteria in Baltic Sea are smaller than 1 μm, however, the largest ones could grow up to 3 μm. Albertano *et al.* (1997) separated three classes of their size: the largest cell fraction from 1.1 to 2.9 μm, medium from to approximately 25% of those found in natural environments due to a very high nutrient concentration in the medium (Stal *et al.* 2003).

Irradiance, temperature, and nutrients are major factors controlling growth, photosynthetic activity, and distribution of autotrophic picoplankton (Jasser and Arvola 2003). These factors determine considerably the abundance of marine *Synechococcus* community (Jasser and Arvola 2003, Stal *et al.* 2003, Jasser 2006). In earlier studies, Jodłowska *et al.* (2010) described the influence of light intensity on the growth and pigment composition of *Synechococcus* strains. Cellular concentration of each investigated strains was significantly highest at low light intensity [10 μmol(photon) m^{-2} s^{-1}]. In general, in 3 cyanobacterial strains tested, cellular Chl *a*, phycoerythrin, and phycocyanin content were negatively affected by irradiance, except for phycocyanin in BA-120, the content of which did not differ significantly in the whole range of irradiances tested. In this work, elevated irradiance had a negative effect on cell-specific P_{max} and α, and Chl fluorescence in each of the studied *Synechococcus* strains. The highest values of these parameters were noted at the lowest light intensity. Most cyanobacteria prefer low light intensity for growth and photosynthesis (Fogg and Thake 1987, Ibelings 1996). This statement is consistent with observations of autotrophic picoplankton maximum abundance at or near the base of euphotic zone in coastal and offshore marine waters (Glover *et al.* 1985, Fogg 1986, Stal *et al.* 2003, Callieri *et al.* 2005). Under cultivation conditions, some major picoplankters could survive and resume growth after periods of total darkness. This feature allows them to withstand seasonal rhythm of winter darkness and sinking into the aphotic zone (Antia 1976). However, Kana and Glibert (1987) showed that *Synechococcus* WH7803 can also grow at irradiance as high as 2,000 μmol(photon) m^{-2} s^{-1}, but only if the cultivation of the strain was preceded by the acclimation under several intermediate irradiances.

Synechococcus strains were tolerant to elevated light and high capacity of acclimation to irradiance and temperature. These strains were able to adjust their composition of photosynthetic pigments to use light quanta more effectively and to protect themselves from unfavorable effect of excessive light (Jodłowska *at al.* 2010). Moreover, alteration of the pigment content and their ratios optimize photosynthetic efficiency (Richardson *et al.* 1983, MacIntyre *et al.* 2002, Defew *et al.* 2004, Dubinsky and Stambler 2009). In changeable, light-limited waters, *Synechococcus* strains benefit from their ability to maintain their optimal growth rate under low light conditions and from potentially low photoinhibition under exposure to high light intensities (Jasser 2006).

In photoautotrophs acclimated to high light, lower cellular content of Chl *a* is related to a decrease in a size and/or a number of PSU, which is reflected by *P-I* curves (Platt *et al*. 1980, Prézelin 1981, Ramus 1981, Richardson *et al*. 1983, Henley 1993, Dring 1998, Mouget *et al*. 1999,

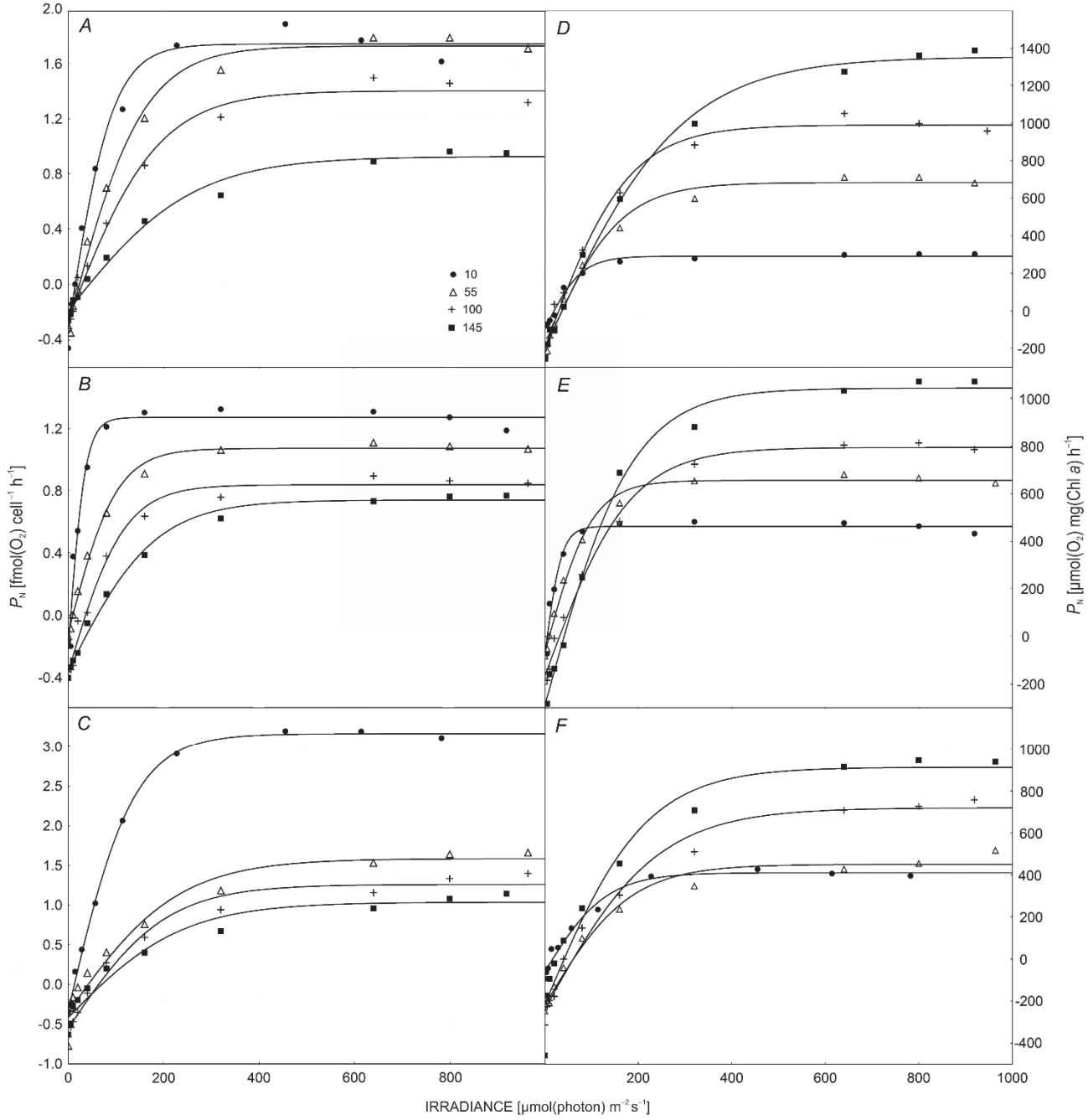

Fig. 2. Net photosynthetic rate (P_N) [fmol(O_2) cell $^{-1}$ h^{-1}] in *Synechococcus* strains: (*A*) BA-120, (*B*) BA-124, (*C*) BA-132, and P_N [µmol(O_2) mg(Chl *a*) h^{-1}] in (*D*) BA-120, (*E*) BA-124, (*F*) BA-132 growing at different irradiances and temperature of 22.5°C. Mean ± SE (n = 3).

MacIntyre *et al.* 2002, Dubinsky and Stambler 2009, Jodłowska and Latała 2010, 2012). The results for *Synechococcus* strains show that this cyanobacteria conforms to more than one of photoadaptive models used to categorize species (Prézelin 1981, Richardson *et al*. 1983).

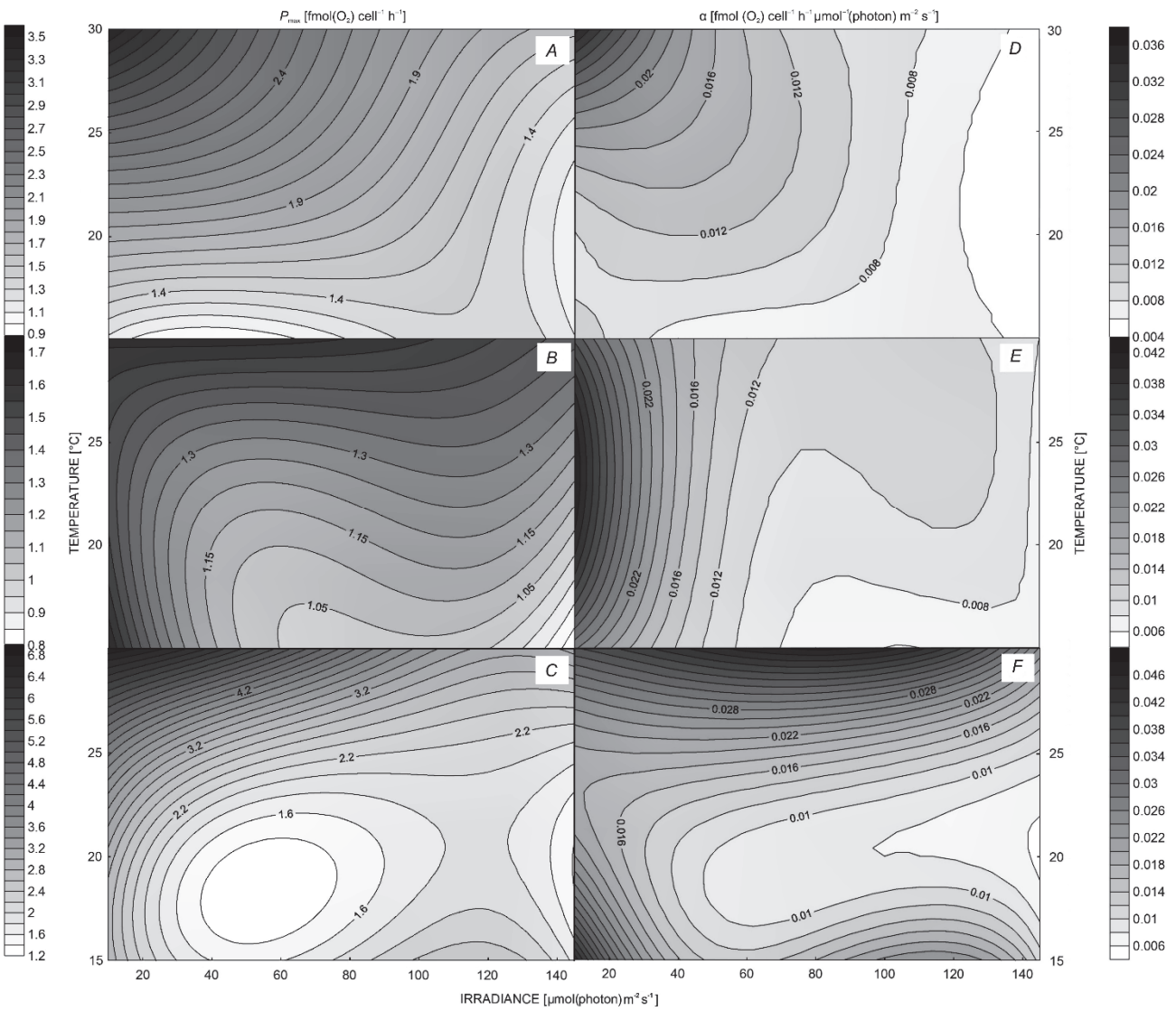

Fig. 3. Response-surface estimation of cell-specific maximum photosynthetic rate (P_{max}) in *Synechococcus* strains: (*A*) BA-120, (*B*) BA-124, (*C*) BA-132 and cell-specific initial slope of photosynthetic light response curve (α) in strains: (*D*) BA-120, (*E*) BA-124, (*F*) BA-132 after 14 d of cultivation at different temperatures [°C] and irradiances [µmol(photon) m^{-2} s^{-1}]

In strains acclimated to low light rather than those grown in the high light, higher cell-specific P_{max} might indicate change in the number of PSU. However, the change in the size of PSU might be indicated by higher Chl *a*-specific P_{max} in high-light strains in comparison to those under low light. The α parameter, calculated per cell, rises under low light due to improved light utilization efficiency. Similarly, cell-specific P_{max} is affected by photoacclimation and may be even 2-fold higher in low light-acclimated cultures than in their high-light counterparts. This difference correlated closely with amount of Rubisco per PSU (Dubinsky and Stambler 2009). Photoacclimation process involves massive changes in cellular content of both light-harvesting and photoprotective pigments and it is also reflected in major adjustment in the ultrastructure of phytoplankton. There is always the increase in the number of thylakoids under low light, resulting from a total membrane area increase required to accommodate added pigment molecules (Dubinsky and Stambler 2009). In the present study, direct observation of thylakoid ultrastructure confirmed the influence of light intensity on amount of thylakoid membranes. However, we could only prove the existence of photoacclimation mechanism that involves a change in the number of PSU, but we could not confirm changes in their size.

Fluorescence parameters, F_V/F_M and Φ_{PSII}, provide additional information concerning the photosynthetic apparatus (Maxwell and Johnson 2000, Roháček 2002).

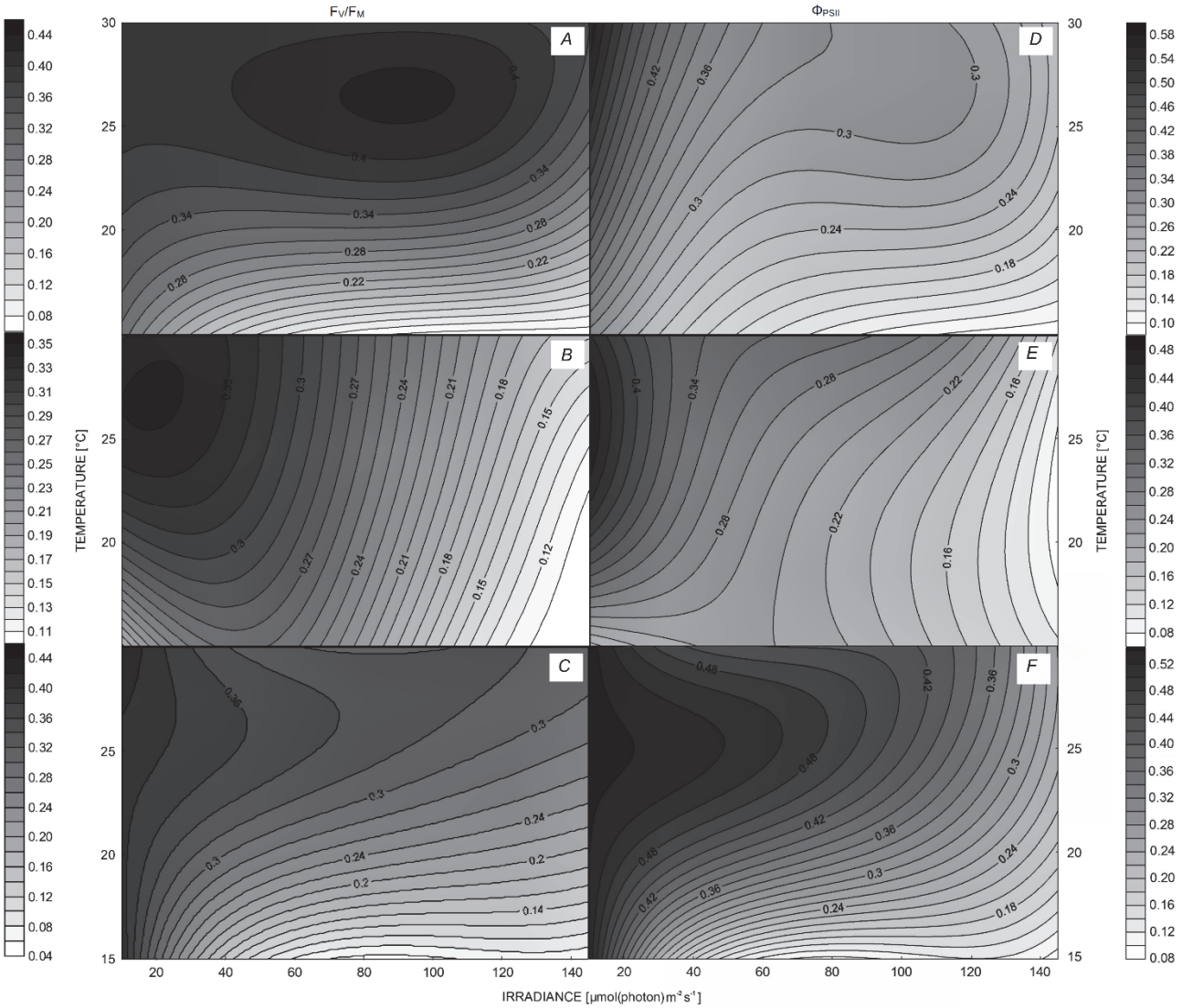

Fig. 4. Response-surface estimation of maximum quantum yield of PSII photochemistry (F_V/F_M) in *Synechococcus* strains: (*A*) BA-120, (*B*) BA-124, (*C*) BA-132, and effective quantum yield of PSII photochemistry (Φ_{PSII}) in: (*D*) BA-120, (*E*) BA-124, (*F*) BA-132 after 14 d of cultivation at different temperatures [°C] and irradiances.

However, interpretation of fluorescence should take into account in cyanobacteria that the principal light-harvesting complexes are phycobilisomes located peripherally to the thylakoid membranes and cellular phycobiliprotein content influences cellular fluorescence yield. In higher plants, F_V/F_M is near 0.8 under optimal conditions and lower values reflect inhibition of PSII function (Björkman and Demmig 1987). In a *Synechococcus* sp. PCC 7942 mutant, lacking phycocyanin, F_V/F_M was about 0.75 under acclimated growth. However, the values of 0.4 to 0.6 were observed in wild-type *Synechococcus* grown under the same conditions (Campbell *et al.* 1998). Reduced values of F_V/F_M partly due to an increased value of F_0 fluorescence, which in turn is caused by emissions from PSII, phycobiliproteins, and possible also PSI Chl. In present study, values of F_V/F_M and Φ_{PSII} were also low and they changed in the ranges of 0.04–0.44 and 0.08–0.58, respectively, depending on light intensities. Changes observed in fluorescence might be due to the different pigment composition. In cyanobacteria with the constant pigment content, changes in F_V/F_M correlate well with changes in PSII-related function, such as oxygen evolution, but the absolute value of F_V/F_M is not the reliable indicator of PSII function (Campbell *et al.* 1998). Thus, we must approach cautiously the results of both F_V/F_M and Φ_{PSII} depending on light-intensity changes. In the earlier studies on *Synechococcus* strains, Jodłowska *et al.* (2010) observed that a ratio of phycobilins to Chl *a* was constant across the whole range of light intensities and a cellular carotenoid content increased with an increase in irradiance. In high light, *Synechococcus* strains are challenged to maintain high photosynthetic efficiency and

simultaneously prevent photodamage that results from low level of electron acceptors downstream of PSII (Mackey *et al.* 2008). Moreover, in high light, *Synechococcus* strains showed high cellular carotenoid content (Jodłowska *et al.* 2010). It might also reduce the amount of absorbed light quanta used in photochemical processes because of excess energy dissipation .

Both prokaryotic and eukaryotic species can successfully acclimate to higher irradiances (Kana and Glibert 1987, Mouget *et al.* 1999, Jodłowska and Latała 2010, Jodłowska and Latała 2012). However, eukaryotic species showed a higher photoacclimation potential than prokaryotic ones when compared under identical experimental conditions relevant for the open ocean. Differences were observed both in growth and photosynthetic parameters (Kulk *et al.* 2011). Authors suggested that a presence of xanthophyll cycle might play an important role in successful photoacclimation of eukaryotic phytoplankton species. Nevertheless, prokaryotic phytoplankton species contain photoprotective, xanthophyll pigment, zeaxanthin (Kulk *et al.* 2011, Jodłowska and Latała 2013). However, its photoprotective role in cyanobacteria is questionable because zeaxanthin is spatially separated from the photosystems. Some previous observations have shown that the zeaxanthin content rises with increasing irradiance providing some form of photoprotection (Jodłowska and Latała 2010, Kulk *et al.* 2011).

In conclusion, irradiance and temperature are main factors inducing changes in Chl *a* fluorescence and photosynthetic rate in three strains of Baltic *Synechococcus*. They may play a dominant role in the ecosystem of Baltic Sea during summer period. *Synechococcus* strains presented similar ecophysiological properties and demonstrated their tolerance to elevated light and temperature, which allowed them to acclimate effectively to varying water systems. Cyanobacteria developed the array of interrelated cellular mechanisms to optimize their light harvesting and utilization when exposed to spatial and temporal irradiance variations.

References

Albertano, P., Di Somma, D., Capucci, E.: Cyanobacterial picoplankton from the Central Baltic Sea: cell size classification by image-analyzed fluorescence microscopy. – J. Plankton Res. **19**: 1405-1416, 1997.

Antia, N.J.: Effects of temperature on the darkness survival of marine microplanktonic algae. – Microb. Ecol. **3**: 41-54, 1976.

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. – Planta **170**: 489-504, 1987.

Blackburn, S.I., McCausland, M.A., Bolch, C.J.S., Newman, S.J., Jones, G.J.: Effect of salinity and toxin production in cultures of the bloom-forming cyanobacterium *Nodularia spumigena* from Australian waters. – Phycologia **35**: 511-522, 1996.

Callieri, C., Moro, S., Caravati, E., Crosbie, N.D., Weisse, T.: Strain-specific photosynthetic response of freshwater picocyanobacteria. – Verh. Internat. Verein. Limnol. **29**: 777-782, 2005.

Campbell, D., Hurry, V., Clarke, A.K., Gustafsson, P., Öquist, G.: Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. – Microbiol. Mol. Biol. R. **62**: 667-683, 1998.

Defew, E.C., Perkins, R.G., Paterson, D.M.: The influence of light and temperature interactions on a natural estuarine microphytobenthic assemblage. – Biofilms **1**: 21-30, 2004.

Dring, M.J.: The Biology of Marine Plants. 199 pp. Cambridge Univ. Press, Cambridge 1998.

Dubinsky, A., Stambler, N.: Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications. – Aquat. Microb. Ecol. **56**: 163-176, 2009.

Fisher, R.A., Yates, F.: Statistical Tables for Biological, Agricultural and Medical Research. 6th ed. 138 pp. Olivier and Boyd, Edinburgh 1963.

Fogg, G.E.: Ocean ecology: Light and Ultraphytoplankton. 99 pp. Nature Publishing Group, London 1986.

Fogg, G.E., Thake, B.: Algal Cultures and Phytoplankton Ecology. 269 pp. University of Wisconsin Press, Madison and Milwaukee 1987.

Glover, H. E., Phinney, D.A., Yentsch, C.S.: Photosynthetic characteristics of picoplankton compared with those of larger phytoplankton populations, in various water masses in the Gulf of Maine. – Biological Oceanogr. **3**: 223-248, 1985.

Hajdu, S., Höglander, H., Larsson, U.: Phytoplankton vertical distributions and composition in Baltic Sea cyanobacterial blooms. – Harmful Algae **6**: 189-205, 2007.

Henley, W.J.: Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. – J. Phycol. **29**: 729-739, 1993.

Ibelings, B.W.: Changes in photosynthesis in response to combined irradiance and temperature stress in cyanobacterial surface waterblooms. – J. Phycol. **32**: 549-557, 1996.

Jasser, I.: The relationship between autotrophic picoplankton (APP) - the smallest autotrophic component of food web and the trophic status and depth of lakes. – Ecohydrol. Hydrobiol. **6**: 69-77, 2006.

Jasser, I., Arvola, L.: Potential effects of abiotic factors on the abundance of autotrophic picoplankton in four boreal lakes. – J. Plankton Res. **25**: 873-883, 2003.

Jodłowska, S., Śliwińska, S., Latała, A.: The influence of irradiance on the growth and photosynthetic pigments of three Baltic picocyanobacterial strains of Synechococcus. – In: Olańczuk-Neymen, K., Mazur-Marzec, H. (ed.): Microorganisms in the Environment and Environmental Engineering from Ecology to Technology. Pp. 85-92. Monografie Komitetu Inżynierii Środowiska PAN, no. 64, Zabrze 2010.

Jodłowska, S., Latała, A.: Photoacclimation strategies in toxic cyanobacterium *Nodularia spumigena* (Nostocales, Cyanobacteria) – Phycologia **49**: 203-211, 2010.

Jodłowska, S., Latała, A.: Mechanisms of photoacclimation on photosynthesis level in Cyanobacteria. – In: Najafpour, M.M. (ed.): Advances in Photosynthesis - Fundamental Aspects. Pp. 97-108. InTech, Croatia, 2012.

Jodłowska, S., Latała, A.: Combined effects of light and temperature on growth, photosynthesis, and pigment content in the mat-forming cyanobacterium *Geitlerinema amphibium*. – Photosynthetica **51**: 202-214, 2013.

Kahru, M., Leppänen, J.-M., Rud, O., Savchuk, O.P.: Cyano-

bacterial blooms in the Gulf of Finland triggered by saltwater inflow into the Baltic Sea. – Mar. Ecol. Prog. Ser. **207**: 13-18, 2000.

Kana, T.M., Glibert, P.M.: Effect of irradiances up to 2000 μmol E m^{-2} s^{-1} on marine *Synechococcus* WH7803-I. Growth, pigmentation, and cell composition. – Deep-Sea Res. **34**: 479-495, 1987.

Kulk, G., van de Poll, W.H., Visser, R.J.W, Buma, A.G.J.: Distinct differences in photoacclimation potential between prokaryotic and eukaryotic oceanic phytoplankton. – J. Exp. Mar. Biol. Ecol. **398**: 63-72, 2011.

Latała, A., Jodłowska, S., Pniewski, F.: Culture Collection of Baltic Algae (CCBA) and characteristic of some strains by factorial experiment approach. – Algological Studies, **122**: 137-154, 2006.

Mackey, K.R.M., Paytan, A., Grossman, A.R., Bailey, S.: A photosynthetic strategy for coping in a high light, low nutrient environment. – Limnol. Oceanogr. **53**: 900-913, 2008.

MacIntyre, H.L., Kana, T.M., Anning, T., Geider, R.J.: Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and Cyanobacteria. – J. Phycol. **38**: 17-38, 2002.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Mouget, J.-L., Tremblin, G., Morant-Manceau, A., Morancais, M., Robert, J.-M.: Long-term photoacclimation of *Haslea ostrearia* (Bacillariophyta): effect of irradiance on growth rates, pigment content and photosynthesis. – Eur. J. Phycol. **34**: 109-115, 1999.

Oktaba, W.: [Methods of Mathematical Statistics in Experiments]. 488 pp. PWN, Warszawa 1986. [In Polish]

Panosso, R., Graneli, E.: Effects of dissolved organic matter on the growth of *Nodularia spumigena* (Cyanophyceae) cultivated under N or P deficiency. – Mar. Biol. **136**: 331-336, 2000.

Platt, T., Jassby, A.D.: The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton. – J. Phycol. **12**: 421-430, 1976.

Platt. T, Gallegos, C.L., Harrison, W.G.: Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. – J. Mar. Res. **38**: 687-701, 1980.

Prézelin, B.B.: Light reactions in photosynthesis. – Can. B. Fish. Aquat. Sci. **210**: 1-43, 1981.

Ramus, J.: The capture and transduction of light energy. – In: Lobban, C.S., Wynne, M.J. (ed.): The Biology of Seaweeds. Pp. 458-492. Blackwell Scientific, Oxford 1981.

Richardson, K., Beardall, J., Raven, J.A.: Adaptation of unicellular algae to irradiance: an analysis of strategies. – New Phytol. **93**: 157-191, 1983.

Richardson, T.L., Jackson, G.A.: Small phytoplankton and carbon export from the surface ocean. – Science **315**: 838-840, 2007.

Rohacek, K.: Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. – Photosynthetica **40**: 13-29, 2002.

Six, C., Finkel, Z.V., Irwin, A.J., Campbell, D.A.: Light variability illuminates niche-partitioning among marine picocyanobacteria. – PLOS ONE: doi:10.1371/journal.pone. 0001341, 2007.

Snedecor, G.W., Cochran, W.G.: Statistical Methods. 703 pp. Iowa State University Press, Ames 1980.

Stal, L.J., Walsby, A.E.: Photosynthesis and nitrogen fixation in a cyanobacterial bloom in the Baltic Sea. – Eur. J. Phycol. **35**: 97-108, 2000.

Stal, L.J., Albertano, P., Bergman, B., *et al*.: BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea – responses to a changing environment. – Cont. Shelf Res. **23**: 1695-1714, 2003.

Stanier, R. Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G.: Purification and properties of unicellular blue-green algae (order Chroococcales). – Bacteriol. Rev. **35**: 171-205, 1971.

Strickland, I.D.H., Parsons, T.R.: A practical handbook of seawater analysis. – Bull. Fish. Res. Bd. Can. **167**: 1-310, 1972.

Surosz, W., Palinska, K.A.: Ultrastructural changes induced by selected Cd and Cu concentrations in the cyanobacterium *Phormidium*: Interaction with salinity. – J. Plant Physiol. **157**: 643-650, 2000.