Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 47, Issue 2, June 2018
pages (202-210)

ISSN 1730-413X
eISSN 1897-3191

Photosynthetic efficiency of endosymbiotic algae of *Paramecium bursaria* originating from locations with cold and warm climates

by

Katarzyna Możdżeń[1,*], Patrycja Z. Leśnicka[1], Tomasz Burnecki[1], Sylwia Śliwińska-Wilczewska[2], Andrzej Skoczowski[1], Magdalena Greczek-Stachura[1]

DOI: **10.1515/ohs-2018-0019**
Category: **Short communication**
Received: **September 7, 2017**
Accepted: **November 21, 2017**

[1]*Department of Plant Physiology, Institute of Biology, Pedagogical University of Cracow, ul. Podchorążych 2, 30-084 Kraków, Poland*

[2]*Department of Marine Ecosystem Functioning, Institute of Oceanography, Faculty of Oceanography and Geography, University of Gdańsk, Al. M. Piłsudskiego 46, 81-378 Gdynia, Poland*

* Corresponding author: *kasiamozdzen@interia.pl*

Abstract

Paramecium bursaria (Ciliophora) is a cosmopolitan unicellular organism that plays a significant role in aquatic ecosystems. *P. bursaria* contains symbiotic algae and this association is a mutual symbiosis. The aim of the present study was to determine the activity of photosystem II (PSII) in *Chlorella* sp. inside *P. bursaria* cells. Ciliates were incubated for 7 days at different temperatures from 6 to 18°C, under the circadian cycle: 12 h light/12 h dark, at light intensity of 200 µmol m^{-2} s^{-1} and under constant darkness conditions. The control group was kept at a temperature of 18°C under constant light conditions. Changes in PSII were monitored using different fluorescence parameters. Differences in responses between endosymbiotic algae of two *P. bursaria* strains – Ard7 from a warm climate and KD64 from a cold climate – were determined. The highest photosynthetic activity of *P. bursaria* green endosymbionts was observed at a temperature of 18°C, regardless of the light conditions. Algae from warm climate were more sensitive to cold temperature stress than algae from *P. bursaria* collected in cold climate.

Key words: chlorophyll *a* fluorescence, endosymbiotic *Chlorella* sp., photosystem II (PSII), temperature

DE GRUYTER

The *Oceanological and Hydrobiological Studies* is online at oandhs.ocean.ug.edu.pl

Introduction

Paramecium bursaria (Ehrenberg 1831) is a unicellular organism belonging to ciliates that play a significant role in aquatic ecosystems. Ciliates are considered to be cosmopolitan. They can be easily found in various types of watercourses and bodies of stagnant water. The cytoplasm of a single cell of *P. bursaria* contains about 700 symbiotic algal cells representing different species (Hoshina, Imamura 2009). Algae cells containing green photosynthetic pigments (chlorophyll *a* and *b*) provide protozoan with products of photosynthesis: maltose and oxygen and, in return, they receive carbon dioxide and nitrogen compounds (Reisser 1980). Moreover, *P. bursaria* protects algal cells from other protozoans and viruses, and directs them to brightly illuminated areas for optimum photosynthesis conditions (Yamada et al. 2006; Reisser 1980).

The growth of photosynthetic organisms is a dynamic process continuously shaped by environmental conditions. The most rapidly changing environmental factor affecting the photosynthesis is light which is a source of energy for carbon fixation (Gilstad et al. 1993; Flameling, Kromkamp 1997; Walters, Horton 1995). During the day, the quality and quantity of photosynthetically active radiation (PAR) changes. Photosynthetic organisms maintain a balance between the photosynthetic energy supply in thylakoid membranes and the energy consumption within the Calvin cycle (Carvalho et al. 2011; Bailey et al. 2008). When algae and some species of higher plants are moved from light to dark conditions, the PSII activity is reduced, probably due to the low rate of reduction of primary electron acceptors (Dau 1994). Furthermore, when the light intensity is too high, the photosynthetic apparatus can be damaged due to the chlorophyll photooxidation.

Photobiological responses and adaptations of *P. bursaria* endosymbionts exposed to PAR have become an important research subject (Berk et al. 1991; Summerer et al. 2009). Pado (1965) showed that light conditions determine the number of algal cells inside the host cell.

Chlorophyll fluorescence provides information about the physiological state of the organism, the amount of energy absorbed by the chlorophyll molecule and dissipated in the form of heat (Murchie, Lawson 2013). Stress factors lead to increased power dissipation in the PSII center and may in time lead to irreversible damage. Changes in chlorophyll *a* fluorescence provide insight into the processes of photoprotection and photodamage with respect to the reduction of photosynthesis efficiency (Franklin et al. 1992).

Interactions between temperature and light have a significant impact on photosynthesis and the growth rate of algae (Butterwick et al. 2005). Low temperature may induce structural and functional changes in the photosynthetic apparatus and RuBisCo activity allowing to maintain the energy balance (Mortain-Bertrand et al. 1988). Levasseur et al. (1990) showed that a temperature-induced decrease in the carbon fixation rate was correlated with a decrease in the energy-transfer efficiency between the antenna and the reaction center of PSII. Thompson et al. (1992) reported that the chlorophyll content in algae decreases with decreasing temperature. Measurement of chlorophyll *a* fluorescence of *Chlorella vulgaris* incubated at 5°C indicated that the yield of PSII electron transport was less temperature sensitive than in organisms incubated at 2°C (Maxwell et al. 1994). Under the culture conditions, the temperature should oscillate between 20 and 30°C. Temperature lower than 16°C can slow down the growth of algae and temperature higher than 35°C can be lethal to many

Table 1

Chlorophyll *a* fluorescence parameters

Technical fluorescence parameters	
F_0	Chlorophyll fluorescence intensity measured when all photosystem II reaction centers are open
F_m	Maximal chlorophyll fluorescence intensity measured when all photosystem II reaction centers are closed
F_v	Variable chlorophyll fluorescence (F_m/F_0)
F_v/F_m	Maximum quantum yield of PSII
F_v/F_0	Efficiency of the water-splitting complex on the donor side of PSII
DIo/CSo	Dissipated energy flux per cross section (CS) at t = 0
ETo/CSo	Electron transport flux per cross section (CS) at t = 0
PI	Indicator of the PSII functioning
RC/ABS	Index expression as the density of reaction centers (RC)
$Tf_{(max)}$	Time needed to reach F_m (ms)
TRo/CSo	Trapped energy flux per cross section (CS) at t = 0

DE G DE GRUYTER

strains of algae (Coutteau 1996). According to Murata et al. (2007), low temperature stress inhibits the repair of PSII but does not affect the photodamage to PSII. Many studies describe effects of short- and long-term exposure to low temperature on the photosynthetic activity of algae (Morgan-Kiss et al. 2002; Wen et al. 2005). The symbiotic *Chlorella* of *P. bursaria* differs significantly from free-living algae at the higher activity of RuBisCo, regardless of the CO_2-concentration and carbonic anhydrase activity (Reisser, Benseler 1981).

The aim of the present study was to determine the efficiency of the photosynthetic apparatus of *P. bursaria* symbiotic *C. vulgaris* by measuring the chlorophyll *a* fluorescence kinetics under low-temperature and constant dark stress. Differences in responses between strain Ard7 and KD64 were determined. Ard7 is originated from Ardmore, OK (USA) with an annual average temperature of 18°C, 235 days of sunshine per year and annual precipitation of 930 mm and strain KD64 originated from Kamchatka with an annual average temperature of 1.6°C, 67 days of sunshine per year and annual precipitation of 2700 mm.

Materials and methods

Paramecium bursaria strains cultivation

Green strains of *Paramecium bursaria* (Ard7 and KD64) were collected from Ardmore, OK (USA) and Kamchatka (Russia), respectively. They were maintained at the Culture Collection of Ciliates and their Symbionts of St. Petersburg University. Paramecia were cultivated on a lettuce medium according to Sonneborn (1970). The control samples were incubated at a temperature of 18°C in the daily cycle of 12 light hours and 12 dark hours (12L/12D) in climate chambers (Angelantoni Life Science, Italy). The experimental groups were incubated for 7 days at different temperatures (18, 15, 12, 9, and 6°C) in constant dark (24D) and light/dark (12L/12D) conditions. The light intensity was 200 µmol m^{-2} s^{-1} by using a quantum sensor (model 189, Li-Cor, Inc, Lincoln, USA).

Chlorophyll fluorescence

The photosynthetic activity of symbionts was measured by chlorophyll *a* fluorescence using the Plant Efficiency Analyzer (Handy-PEA fluorimeter, Hansatech Instruments Ltd, Pentney, King's Lynn, Norfolk, England). *P. bursaria* samples were collected directly from the culture tubes, placed in 2 ml glass cuvettes in the liquid adapter Handy-PEA and adapted to dark for 20 minutes. Then, the samples were illuminated with a high-intensity of the excitation light, 3 µmol m^{-2} s^{-1}, and the parameters were measured within 10 µs–30 ms. The basic and specific fluorescence parameters are presented in Table 1.

Statistical analysis

The results were presented as mean values for 3 independent replicates. Statistical significance between mean values was assessed by Duncan's multiple range test using STATISTICA 10.0 statistical software (StatSoft Inc.). A probability of $p < 0.05$ was considered as statistically significant.

Results and discussion

The results obtained in the present study revealed significant changes in PSII in algae strains incubated at 18°C compared to algae from lower temperatures in the light and dark conditions.

The maximum quantum yield of PSII (F_v/F_m) is used to estimate changes in the functioning of reaction centers, as well as to determine the photosynthetic activity (Büchel, Wilhelm 1993). According to Masojídek et al. (2011), the PSII efficiency varies depending on the time of day, which is related to the intensity of light. The photoinhibition is induced not only due to the excess of unused energy by chlorophyll (Osmond et al. 1999), but also by low temperature stress (Germino, Smith 2000a,b). The research conducted by Gómez et al. (2001) revealed that *Gelidium pulchellum* in high light showed a greater photoinhibition ratio and slower ability to rebuild structures of PSII compared to plants growing under low light conditions. In the present study, the reduction in the F_v/F_m parameter was observed with decreasing temperature regardless of the light conditions. The highest F_v/F_m values were determined in cultures kept under 18°C and 12L/12D conditions. This parameter had the lowest values compared to the control, regardless of the light conditions at a temperature of 6°C (Table 2). Zhang et al. (2012) reported that F_v/F_m of cyanobacteria and green algae was higher at a temperature of 15°C than at 4°C. According to Teoh et al. (2013), the optimal temperature for the growth of *Chlorella* strains is 18°C. On the other hand, Wilson & Huner (2000) showed that F_v/F_m in *C. vulgaris* incubated at 5°C under low light intensity was higher than at a temperature of 27°C. In this study, the F_v/F_m values of *P. bursaria* strains incubated under dark conditions did not change significantly (Table 2). Changes in F_v/F_m result from the

Table 2

Some chlorophyll *a* fluorescence parameters of two strains (Ard7 and KD64) of *Paramecium bursaria* incubated in temperatures (T): 18, 15, 12, 9, 6°C in the daily cycle of 12 light hours and 12 dark hours (12L/12D), and in constant darkness (24D) conditions

Treatment		Endosymbionts of *Paramecium bursaria*									
		Ard7					KD64				
T(°C)	Light conditions	F_0	F_m	F_v	F_v/F_m	F_v/F_0	F_0	F_m	F_v	F_v/F_m	F_v/F_0
18	12L/12D	161.00^{c}	348.30^{b}	187.30^{a}	0.54^{a}	1.16^{a}	45.30^{c}	105.70^{b}	60.30^{a}	0.58^{a}	1.33^{ab}
	24D	192.30^{b}	312.00^{c}	119.70^{c}	0.39^{bc}	0.62^{bcd}	63.30^{b}	99.30^{c}	36.00^{c}	0.36^{cd}	0.57^{bc}
15	12L/12D	241.00^{a}	415.70^{a}	174.70^{b}	0.42^{b}	0.72^{bcd}	71.70^{a}	119.70^{a}	48.00^{b}	0.40^{bcd}	0.67^{abc}
	24D	91.30^{d}	162.00^{d}	70.70^{d}	0.44^{b}	0.77^{bc}	37.70^{d}	58.70^{d}	21.00^{d}	0.36^{cd}	0.56^{bc}
12	12L/12D	70.00^{e}	105.00^{e}	35.30^{e}	0.34^{c}	0.50^{cd}	39.00^{d}	59.00^{d}	20.00^{d}	0.34^{d}	0.51^{bc}
	24D	40.30^{f}	68.30^{f}	28.00^{ef}	0.41^{bc}	0.69^{bcd}	37.70^{d}	58.70^{d}	21.00^{d}	0.36^{cd}	0.56^{bc}
9	12L/12D	34.30^{g}	59.70^{f}	25.30^{f}	0.42^{b}	0.74^{bcd}	27.30^{e}	40.70^{e}	13.30^{e}	0.33^{d}	0.49^{bc}
	24D	25.30^{h}	40.70^{g}	15.30^{g}	0.38^{bc}	0.60^{bcd}	20.30^{f}	29.00^{f}	8.70^{f}	0.30^{d}	0.43^{c}
6	12L/12D	5.30^{j}	9.70^{h}	4.30^{h}	0.45^{b}	0.83^{b}	10.70^{g}	16.30^{g}	5.70^{g}	0.35^{cd}	0.55^{bc}
	24D	11.00^{i}	16.30^{h}	5.30^{h}	0.33^{c}	0.49^{d}	4.70^{h}	8.00^{h}	3.30^{g}	0.32^{cd}	0.43^{c}

Different letters indicate significant differences according to Duncan's test ($p < 0.05$). F_0 – chlorophyll fluorescence intensity measured when all photosystem II reaction centers are open, F_m – maximal chlorophyll fluorescence intensity measured when all photosystem II reaction centers are closed, F_v – variable chlorophyll fluorescence (F_m-F_0), F_v/F_m – maximum quantum yield of PSII, F_v/F_0 – efficiency of the water-splitting complex on the donor side of PSII

reduced production of photosynthesis. They depend on protein D1 and photooxidation of PSII (Vass et al. 1992; Krzemińska et al. 2015). The decrease in F_v/F_m may result from the increase in protective non-radiative energy dissipation or photo-inhibitory damage to the PSII reaction center (Maxwell, Johnson 2000). Under changing light intensity and low temperature, the CO_2 fixation slows down, causing the reduction in electron transport. The low temperature inhibits the scavenging of reactive oxygen species (ROS) that may damage PSII and also inhibits the PSII repair cycle (Torzillo et al. 1998).

The decrease in the F_v/F_m ratio under varying light intensity can be due to changes in the level of F_0 and F_m. This indicates the loss of energy dissipated in the form of heat, which may result from the inability of PSII to reduce the complete electron acceptors (Magnusson 1997; Saakov 2002). The F_0 fluctuations include changes at the level of the main antenna as light-harvesting complexes, from the trimeric form to the monomeric form. They also include physical dissociation of the core part of LHCII in PSII. The highest intensity of chlorophyll fluorescence F_0, when all PSII reaction centers are open, was observed under 12L/12D conditions at 15°C in both *P. bursaria* strains. At a temperature of 18°C and 15°C, the F_0 values were significantly higher in Ard7 compared to KD64, which can be due to the adaptation to the optimal temperature of Ard7 originating from a place with a warm climate. When the temperature decreased, the F_0 significantly decreased, regardless of the light conditions. The lowest F_0 values were determined at 6°C (Table 2). F_0 of *Chlorella pyrenoidosa* incubated in dark conditions largely depends on temperature. These reactions indicate a direct effect of thermal shock associated with PSII (Chemeris et al. 2004b). The accumulation of PSII complexes under dark conditions is characterized by low electron reduction, which depends on the reduced plastoquinone. Processes regulating the photosynthetic apparatus include phosphorylation of thylakoid membrane proteins by protein kinases, which reversibly enhance the rate of plastoquinone reduction. These processes include: (i) the energy distribution between PSI and PSII, (ii) the ratio of cyclic and non-cyclic electron transport, (iii) the synthesis and degradation of thylakoid membrane proteins, (iv) the control of PSII reaction center stability, and (v) the deactivation of the main energy bands in thylakoid membranes (Allen et al. 1995). Chemeris et al. (2004a) revealed the close relationship between the state of reduced plastoquinone and the relative content of inactive PSII complexes.

In the present study, the highest F_m value was determined at 15°C under 12L/12D. These significant differences between two strains may be due to different conditions in which they live. The F_m values for the strains kept under 12L/12D were higher compared to the strains incubated in constant darkness. The lowest values of F_m for both strains were observed at 6°C, regardless of the light conditions (Table 2).

Slight differences between F_m and F_0 represent low F_v values, which indicate the low PSII activity and the energy dissipation in the form of heat. This parameter is determined by the maximum quantum yield of PSII. In the present study, the highest F_v was observed

at 18°C and 15°C for Ard7 kept under 12L/12D conditions. The significantly highest F_v values were observed in KD64 incubated under similar light conditions. F_v of symbiotic algae cultured under 24D conditions were statistically lower compared to the cultures grown under 12L/12D conditions. When the temperature decreased, the F_v decreased significantly. The lowest F_v values were determined at a temperature of 6°C under both light conditions (Table 2). It seems that photoinhibition includes at least two inactivation steps. The first phase is considered reversible and develops for about one hour without causing any damage to the PSII (Leitsch et al. 1994). The second stage probably begins with damage in the PSII reaction centers (Aro et al. 1993; Leitsch et al. 1994; Mishra, Singhal 1992; Vass et al. 1992). The highest F_v/F_0 values were noticed in algae strains incubated at a temperature of 18°C, regardless of the light conditions. At temperatures of 12°C, 9°C and 6°C, these values ranged from 0.49 to 0.83 under 12L/12D conditions and from 0.43 to 0.77 under 24D conditions (Table 2). Changes in F_v/F_0 values reveal disturbances in the water dissipating complex, which is very sensitive to environmental changes and determines the resistance of plants (Jiang et al. 2006). Similar changes in F_v/F_0 were observed in barley (Kalaji et al. 2012).

The specific parameters of fluorescence, such as RC/ABS, TRo/CSo, Eto/CSo, DIo/CSo and PI, are used to explain the gradual flow of energy through single PSII reaction centers (Force et al. 2003; Xu et al. 2014). These parameters allow to determine the photosynthetic activity of endosymbiotic algae of *P. bursaria* in conditions of varying temperature and light. In the present study, the lowest values of RC/ABS, TRo/CSo, ETo/CSo, DIo/CSo were reported for both strains incubated at a temperature of 6°C (Fig. 1). The highest RC/ABS values at 15°C in dark conditions (24D) were revealed for both strains compared to other temperatures. TRo/CSo was temperature-dependent. The highest values were observed in the control group for both strains, regardless of the light conditions. When the temperature dropped, the TRo/CSo significantly decreased (Fig. 1). The highest DIo/CSo values compared to the control (18°C) were observed for both strains of *P. bursaria* at a temperature of 15°C under 12L/12D conditions (Fig. 1). A decrease or an increase in the DIo/CSo values indicates that part of the energy is converted into heat (Strasser, Strasser 1995; Force et al. 2003). In the present study, the ETo/CSo values were lower at all temperatures compared to the control (18°C) regardless of the light conditions. The reduced electron transport observed in the leaves of barley plants growing in the shade was probably due to energy being trapped by reaction centers (TRo/CSo) and higher loss of energy in the form of heat (DIo/CSo) (Kalaji et al. 2012). The decrease in TRo/CSo and ETo/CSo values indicates that the active reaction centers are converted into inactive centers, which reduces the efficiency of energy capture.

PI is a biophysical parameter indicating changes in PSII, which is calculated by multiplying ABS/Cs × TRo/Cs × ETo (Strasser, Strasser 1995; Force et al. 2003; Stefanov et al. 2011). PI is one of the fluorescence parameters that provides useful information about the state of the vitality of photosynthetic organisms. The PI values were lower under 12L/12D conditions compared to the control. At a temperature of 15°C, the PI values were similar to the control for Ard7 and higher than the control for KD64 under 24D conditions (Fig. 1). According to Ferrante & Maggiore (2007), however, PSII centers are not damaged at low temperatures but at high temperatures. On the other hand, low temperature may indicate a decrease in the accumulation of electron acceptors and in the activity of PSII reaction centers (Vonshak, Novoplansky 2008). Low temperature stress and light stress have synergistic effects and clearly increase the PSII photodamage (Allakhverdieva, Murataa 2004; Nishiyama et al. 2008). These reactions are similar to those observed in algae exposed to various types of stress, such as high salinity (Vonshak 2002), photoinhibition (Lu, Vonshak 1999; Sonoike et al. 2001), bleaching (Hill et al. 2004) or insecticides (Jena et al. 2012).

The time needed to reach the maximum fluorescence ($Tf_{(max)}$) may be useful to identify early changes in photosynthetic activity. For *P. bursaria* endosymbionts, the longest $Tf_{(max)}$ was recorded for the Ard7 strains compared to the KD64 strain. $Tf_{(max)}$ was significantly shorter under 12L/12D conditions. The lowest $Tf_{(max)}$ was observed in cultures incubated at a temperature of 6°C for both strains (Fig. 1).

In natural aquatic ecosystems, the toxic effect of environmental factors on algae depends on the depth of their occurrence and the temperature conditions (Oukarroum et al. 2012). The temperature differences connected with the light factor affect the photosynthetic activity. In some organisms, these factors cause a decrease in the PSII activity, while in others they do not cause significant changes or they are quickly regenerated. This tolerance may result from various repair mechanisms, including the ability to quench the ROS activity or to produce photoprotective compounds, such as mycosporine amino acids (MAAs) and carotenoids (Teoh et al. 2013).

The time of stressor treatment is the most important factor that affects the photosynthetic activity. The parameters of chlorophyll fluorescence

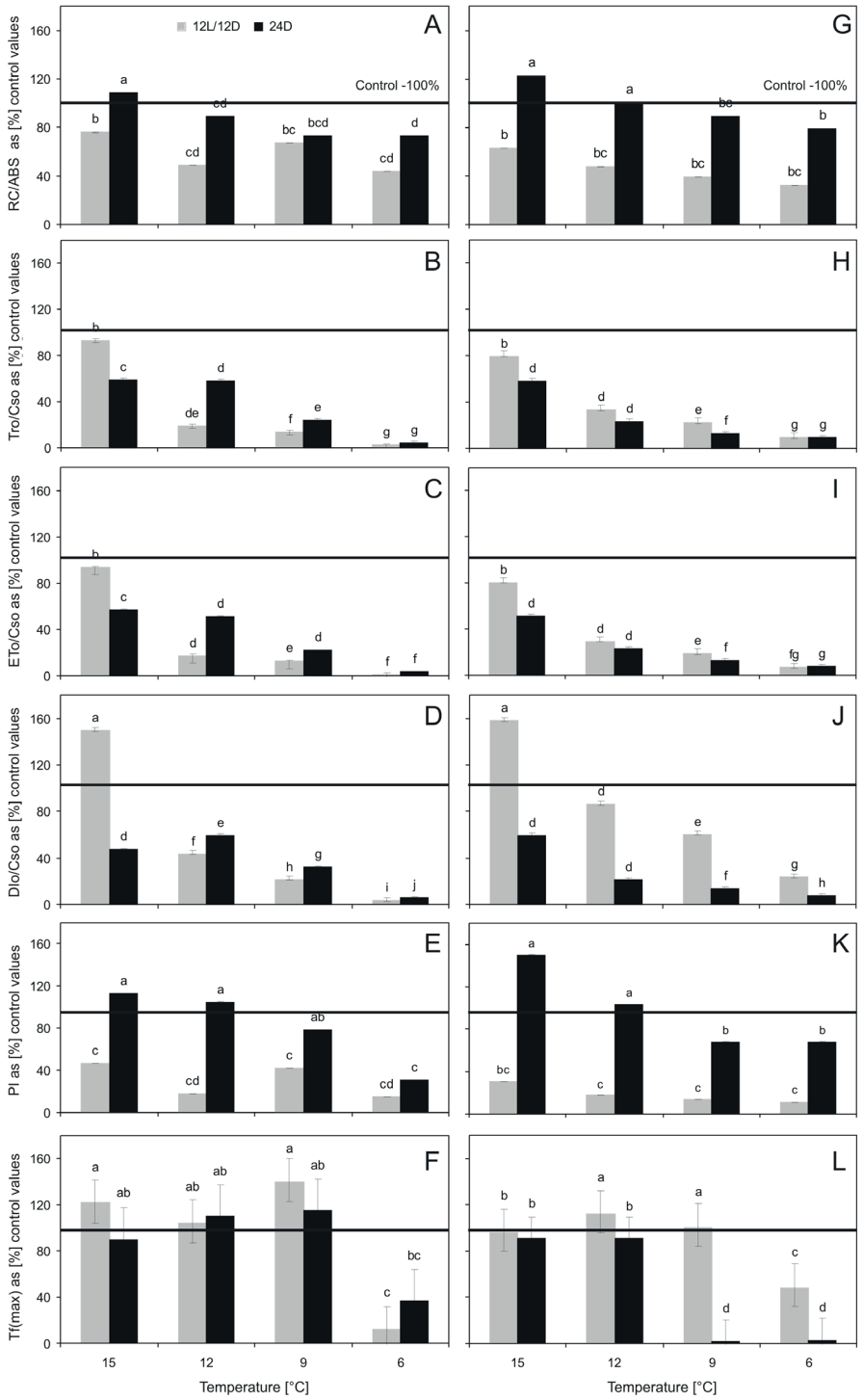

Figure 1

Chlorophyll *a* fluorescence parameters (expressed as a percentage of control – black axis): RC/ABS – index expression as the density of reaction centers (RC), TRo/CSo – trapped energy flux per cross section (CS) at t = 0, ETo/CSo – electron transport flux per cross section (CS) at t = 0, Dlo/CSo – dissipated energy flux per cross section (CS) at t = 0, Tf(max) – time needed to reach Fm (ms), PI – indicator of PSII functioning in two strains Ard7 (A–F) and KD64 (G–L) *Paramecium bursaria* incubated at temperatures (T): 18, 15, 12, 9, 6°C in the daily cycle of 12 light hours and 12 dark hours (12L/12D), and in constant darkness (24D) conditions. Different letters indicate significant differences according to Duncan's test ($p < 0.05$).

used in the present study allow to visualize early changes in PSII under the influence of temperature and light. The ability of endosymbionts from two *P. bursaria* strains to acclimate to low temperature was observed to improve our understanding of the mechanisms involved in the photosynthetic apparatus. Moreover, these measurements may provide a better insight into the problem of cross adaptation to changing environmental conditions.

Conclusions

Based on the obtained results, we can conclude that all of the chlorophyll fluorescence parameters were related to the decreasing temperature and light conditions. In the case of *Paramecium bursaria*, the strain originating from geographical places with a warm climate was more sensitive to low temperature stress than the strain originating from a place with a cold climate. The darkness had a negative impact on the activity of PSII and prolonged the time needed to reach the high efficiency of photosynthesis.

References

Allakhverdieva, S.I. & Murataa, N. (2004). Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage – repair cycle of photosystem II in *Synechocystis* sp. PCC 6803. *Biochim. Biophys. Acta.* 1657: 23–32. DOI: 10.1016/j.bbabio.2004.03.003.

Allen, J.F., Alexciev, K. & Håkansson, G. (1995). Photosynthesis: Regulation by redox signalling. *Curr. Biol.* 5: 869–872. DOI: 10.1016/S0960-9822(95)00176-X.

Aro, E.M., Virgin, I. & Andersson, B. (1993). Photoinhibition of photosystem II. Inactivation, protein damage and turnover. *Biochim. Biophys. Acta.* 1143: 113–134. DOI: 10.1016/0005-2728(93)90134-2.

Bailey, S., Melis, A., Mackey, K.R., Cardol, P., Finazzi, G. et al. (2008). Alternative photosynthetic electron flow to oxygen in marine *Synechococcus*. *Biochim. Biophys. Acta.* 1777: 269–276. DOI: 10.1016/j.bbabio.2008.01.002.

Berk, S.G., Parks, L.H. & Ting, R.S. (1991). Photoadaptation alters the ingestion rate of *Paramecium bursaria*, a mixotrophic ciliate. *Appl. Environ. Microbiol.* 57: 2312–2316.

Büchel, C. & Wilhelm, C. (1993). *In vivo* analysis of slow chlorophyll fluorescence induction kinetics in algae: progress, problems and perspectives. *Photochem. Photobiol.* 58: 137–48. DOI: 10.1111/j.1751-1097.1993.tb04915.x.

Butterwick, C., Heaney, S.I. & Talling, J.F. (2005). Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. *Freshw. Biol.* 50: 291–300. DOI: 10.1111/j.1365-2427.2004.01317.x.

Carvalho, L., Miller, C.A., Scott, E.M., Geoffrey, A.C., Davies, P.S. et al. (2011). Cyanobacterial blooms: statistical models describing risk factors for national-scale lake assessment and lake management. *Sci. Tot. Environ.* 409: 5353–5358. DOI: 10.1016/j.scitotenv.2011.09.030.

Chemeris, Y.K., Korol'kov, N.S., Seifullina, N.K. & Rubin, A.B. (2004b). Changes in the contents of inactive complexes of photosystem II in *Chlorella* cells incubated in the light and darkness. *Russ. J. Plant Physiol.* 51: 287–293.

Chemeris, Y.K., Shenderova, L.V., Venediktov, P.S. & Rubin, A.B. (2004a). Activation of chlororespiration increases chlorophyll fluorescence yield in *Chlorella* adapted to darkness at high temperature. *Biol. Bull.* 31: 143–150.

Coutteau, P. (1996). Micro-algae. In P. Lavens, P. Sorgeloos (Eds.), *Manual on the production and use of live food for aquaculture* (pp. 7–48). Rome, FAO Fisheries Technical, FAO.

Dau, H. (1994). Molecular mechanisms and quantitive models of variable photosystem II fluorescence. *J. Photochem. Photobiol.* 60: 1–23. DOI: 10.1111/j.1751-1097.1994.tb03937.x.

Ferrante, A. & Maggiore, T. (2007). Chlorophyll *a* fluorescence measurements to evaluate storage time and temperature of *Valeriana* leafy vegetables. *Postharvest Biol. Technol.* 45: 73–80. DOI: 10.1016/j.postharvbio.2007.02.003.

Flameling, I.A. & Kromkamp, J. (1997). Photoacclimation of *Scenedesmus protuberans* (Chlorophyceae) to fluctuating irradiances simulating vertical mixing. *J. Plankton. Res.* 19: 1011–1024. DOI: 10.1093/plankt/19.8.1011.

Force, L., Critchley, C. & Van Rensen, J.J.S. (2003). New fluorescence parameters for monitoring photosynthesis in plants. The effect of illumination on the fluorescence parameters of the JIP-test. *Photosynth. Res.* 78: 17–33. DOI: 10.1023/A:1026012116709.

Franklin, L.A., Levavasseur, G., Osmond, C.B., Henley, W.J. & Ramus, J. (1992). Two components of onset and recovery during photoinhibition of *Ulva rotundata*. *Planta* 186: 399–408. DOI: 10.1007/BF00195321.

Germino, M.J. & Smith, W.K. (2000a). Differences in microsite, plant form, and low-temperature photoinhibition in alpine-plants. *Arct. Ant. Alp. Res.* 32: 388–396. DOI: 10.2307/1552387.

Germino, M.J. & Smith, W.K. (2000b). High resistance to low-temperature photoinhibition in two alpine, snowbank species. *Physiol. Plant.* 110: 89–95. DOI: 10.1034/j.1399-3054.2000.110112.x.

Gilstad, M., Johnsen, G. & Sakshang, E. (1993). Photosynthetic parameters, pigment composition and respiration rates of the marine diatom *Skeletonema costatum* grown in continuous light and a 12:12 light-dark cycle. *J. Plankton. Res.* 15: 939–951. DOI: 10.1093/plankt/15.8.939.

Gómez, I., Figueroa, F.L., Sousa-Pinto, I., Viñegla, B., Perez-Rodriguez, E. et al. (2001). Effects of UV radiation and

temperature on photosynthesis as measured by PAM fluorescence in the red alga *Gelidium pulchellum* (Turner) Kützing. *Bot. Mar.* 44: 9–16. DOI: 10.1515/BOT.2001.002.

Hill, R., Schreiber, U., Gademann, R., Larkum, A.W.D., Kühl, M. et al. (2004). Spatial heterogeneity of photosynthesis and the effect of temperature-induced bleaching conditions in three species of corals. *Mar. Biol.* 144: 633–640. DOI: 10.1007/s00227-003-1226-1.

Hoshina, R. & Imamura, N. (2009). Origins of algal symbionts of *Paramecium bursaria*. In M. Fujishima (Ed.), *Endosymbionts in Paramecium* (pp. 1–29). Springer-Verlag GmbH.

Jena, S., Acharya, S. & Mohapatra, P.K. (2012). Variation in effects off our OP insecticides on photosynthetic pigment fluorescence of *Chlorella vulgaris* Beij. *Ecotox. and Environ. Safe.* 80: 111–117. DOI: 10.1016/j.ecoenv.2012.02.016.

Jiang, C.D., Jiang, G.M., Wang, X., Li, L-H., Biswas, D.K. et al. (2006). Increased photosynthetic activities and thermostability of photosystem II with leaf development of elm seedlings (*Ulmus pumila*) probed by the fast fluorescence rise OJIP. *Environ. Exp. Bot.* 58: 261–268. DOI: 10.1016/j.envexpbot.2005.09.007.

Kalaji, H.M., Carpentier, R., Allakhverdiev, S.I. & Bosa, K. (2012). Fluorescence parameters as early indicators of light stress in barley. *J. Photoch. Photobiol. B.* 112: 1–6. DOI: 10.1016/j.jphotobiol.2012.03.009.

Krzemińska, I., Piasecka, A., Nosalewicz, A., Simionato D. & Wawrzykowski, J. (2015). Alterations of the lipid content and fatty acid profile of *Chlorella prototheocoides* under different light intensities. *Bioresource Technol.* 196: 72–77. DOI: 10.1016/j.biortech.2015.07.043.

Leitsch, J., Schnettger, B., Critchley, C. & Krause G.H. (1994). Two mechanisms of recovery from photoinhibition in vivo: reactivation of photosystem II related and unrelated to D1- protein turnover. *Planta* 194: 15–21.

Levasseur, M.E., Morissette, J.C. & Harrison, P.J. (1990). Effects of long-term exposure to low-temperature on the photosynthetic apparatus of *Dunaliella tertiolecta* (Chlorophyceae). *J. Phycol.* 26: 479–484. DOI: 10.1111/j.0022-3646.1990.00479.x.

Lu, C.M. & Vonshak, A. (1999). Photoinhibition in outdoor *Spirulina platensis* cultures assessed by polyphasic chlorophyll fluorescence transients. *J. Appl. Phycol.* 11: 355–359.

Lu, C.M. & Vonshak, A. (2002). Effect of salinity on photosystem II function in cyanobacterial *Spirulina platensis* cells. *Physiol. Plant.* 114: 405–413.

Magnusson, G. (1997). Diurnal measurements of F_v/F_m used to improve productivity estimates in microalgae. *Mar. Biol.* 130: 203–208.

Masojídek, J., Kopecky, J., Giannelli, L. & Torzillo, G. (2011). Productivity correlated to photobiochemical performance of *Chlorella* mass cultures grown outdoors in thin-layer cascades. *J. Ind. Microbiol. Biotechnol.* 38: 307–317. DOI: 10.1007/s10295-010-0774-x.

Maxwell, D.P., Falk, S., Trick, C.G. & Huner, N.PA. (1994). Growth at low temperature mimics high-light acclimation in *Chlorella vulgaris*. *Plant Physiol.* 105: 535–543.

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

Mishra, R.K. & Singhal G.S. (1992). Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoid lipids. *Plant Physiol.* 98: 1–6.

Morgan-Kiss, R.M., Ivanov, A.G., Williams, J., Khan. M. & Huner, N.P.A. (2002). Differential thermal effects on the energy distribution between photosystem II and photosystem I in thylakoid membranes of a psychrophillic and a mesophillic alga. *Biochim. Biophys. Acta.* 1561: 251–265. DOI: 10.1016/S0005-2736(02)00352-8.

Mortain-Bertrand, A., Descolas-Gros, C. & Jupin, H. (1988). Pathway of dark inorganic carbon fixation in two species of diatoms: influence of light regime and regulator factors on diel variations. *J. Plankton. Res.* 10: 199–217. DOI: 10.1093/plankt/10.2.199.

Murata, N., Takahashi, S., Nishiyama, Y. & Allakhverdiev, S.I. (2007). Photoinhibition of photosystem II under environmental stress. *Biochim. Biophys. Acta* 1767: 414–421. DOI: 10.1016/j.bbabio.2006.11.019.

Murchie, E.H. & Lawson, T. (2013). Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *J. Exp. Bot.* 64(13): 3983–3998. DOI: 10.1093/jxb/ert208.

Nishiyama, Y., Allakverdiev, S. & Murata, N. (2008). Regulation by environmental conditions of the repair of photosystem II in cyanobacteria. In B. Demming-Adams, W.W. Adams III & A.K. Matto (Eds.), *Photoprotection, Photoinhibition Gene Regulation, and Environment* (pp. 193–203). Dordrecht, Springer.

Osmond, B., Schwartz, O. & Gunning, B. (1999). Photoinhibitory printing on leaves, visualised by chlorophyll fluorescence imaging and confocal microscopy, is due to diminished fluorescence from grana. *Aust. J. Plant. Physiol.* 26: 717–724. DOI: 10.1071/PP99077.

Oukarroum, A., Perreault, F. & Popovic, R. (2012). Interactive effects of temperature and copper on photosystem II photochemistry in *Chlorella vulgaris*. *J. Photochem. Photobiol. B* 110: 9–14. DOI: 10.1016/j.jphotobiol.2012.02.003.

Pado, R. (1965). Mutual relations of protozooans and symbiotic algae in *Paramecium bursaria*. The influence of light on the groeth of symbionts. *Folia Biol.* 13: 173–182.

Reisser, W. & Benseler, W. (1981). Comparative studies on photosynthetic enzymes of the symbiotic *Chlorella* from *Paramecium bursaria* and other symbiotic and non-symbiotic *Chlorella* strains. *Arch. Microbiol.* 129(2): 178–180.

Reisser, W. (1980). The metabolic interactions between *Paramecium bursaria* Ehrbg. and *Chlorella* spec. in the

Paramecium bursaria-symbiosis III. The influence of different CO_2 concentrations and of glucose on the photosynthetic and respiratory capacity of the symbiotic unit. *Arch. Microbiol.* 125: 291–293.

Saakov, V.S. (2002). High-temperature stress-related changes in the harmonics F_0, F_m, and F_v of pulse-amplitude modulated fluorescence signals: locating thermal damage in reaction centers of photosystem II. *Dokl. Biochem. Biophys.* 382: 4–9.

Sonneborn, T.M. (1970). Methods in *Paramecium* research. In E.D.M. Prescott (Ed.), *Methods in cell biology* (pp. 241–339). New York, Acad. Press.

Sonoike, K., Hihara, Y. & Ikeuchi, M. (2001). Physiological significance of the regulation of photosystem stoichiometry upon high light acclimation of *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.* 42: 379–84. DOI: 10.1093/pcp/pce046.

Stefanov, D., Petkova, V. & Denev, I.D. (2011). Screening for heat tolerance in common bean (*Phaseolus vulgaris* L.) lines and cultivars using JIP-test. *Sci. Hortic.* 128: 1–6. DOI: 10.1016/j.scienta.2010.12.003.

Strasser, B.J. & Strasser, R.J. (1995). Measuring fast fluorescence transients to address environmental questions: the JIP-test. In P. Mathis (Ed.), *Photosynthesis: From Light to Biosphere* (pp. 977–980). Dordrecht, Kluwer Academic Publishers.

Summerer, M., Sonntag, B., Hörtnagl, P. & Sommaruga, R. (2009). Symbiotic ciliates receive protection against UV damage from their algae: A test with *Paramecium bursaria* and *Chlorella*. *Protist* 60(2): 233–243. DOI: 10.1016/j.protis.2008.11.005.

Teoh, M.L., Phang, S.M. & Chu, W.L. (2013). Response of Antarctic, temperate and tropical microalgae to temperature stress. *J. Appl. Phycol.* 25: 285–297.

Thompson, P.A., Guo, M.X. & Harrison, P.J. (1992). Effects of variation in temperature. I. On the biochemical composition of eight species of marine phytoplankton. *J. Phycol.* 28: 481–488. DOI: 10.1111/j.0022-3646.1992.00481.x.

Torzillo, G., Bernardini, P. & Masojídek, J. (1998). On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina platensis* (cyanobacteria). *J. Phycol.* 34: 504–510. DOI: 10.1046/j.1529-8817.1998.340504.x.

Vass, I., Styring, S., Hundal, T., Koivuniemi, A., Aro, E. et al. (1992). Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced QA species promote chlorophyll triplet formation. *Proc. Natl. Acad. Sci. USA* 89: 1408–1412.

Vonshak, A. & Novoplansky, N. (2008). Acclimation to low temperature of two *Arthrospira platensis* (cyanobacteria) strains involves down-regulation of PSII and improved resistance to photoinhibition. *J. Phycol.* 44: 1071–1079. DOI: 10.1111/j.1529-8817.2008.00546.x.

Walters, R.G. & Horton, P. (1995). Acclimation of *Arabidopsis thaliana* to the light environment: regulation of chloroplast composition. *Planta* 197: 475–481.

Wen, X., Gong, H. & Lu, C. (2005). Heat stress induces an inhibition of excitation energy transfer from phycobilisomes to photosystem II but not to photosystem I in a cyanobacterium *Spirulina platensis*. *Plant Physiol. Bioch.* 43: 389–395. DOI: 10.1016/j.plaphy.2005.03.001.

Wilson, K.E. & Huner, N.P.A. (2000). The role of growth rate, redox-state of the plastoquinone pool and the trans-thylakoid DpH in photoacclimation of *Chlorella vulgaris* to growth irradiance and temperature. *Planta* 212: 93–102.

Wilson, K.E., Król, M. & Huner, N.P.A. (2003). Temperature-induced greening of *Chlorella vulgaris*. The role of the cellular energy balance and zeaxanthin-dependent nonphotochemical quenching. *Planta* 217: 616–627. DOI: 10.1007/s00425-003-1021-8.

Xu, H., Liu, G., Liu, G., Yan, B., Duan, W. et al. (2014). Comparison of investigation methods of heat injury in grapevine (Vitis) and assessment to heat tolerance in different cultivars and species. *BMC Plant Biol.* 14: 156–176. DOI: 10.1186/1471-2229-14-156.

Yamada, T., Onimatsu, H. & van Etten, J.L. (2006). *Chlorella* viruses. *Adv. Virus. Res.* 66: 293–336. DOI: 10.1016/S0065-3527(06)66006-5.

Zhang, M., Yu, Y., Yang, Z. & Kong, F. (2012). Photochemical responses of phytoplankton to rapid increasing-temperature process. *Phycol. Res.* 60: 199–207. DOI: 10.1111/j.1440-1835.2012.00654.x.