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Effects of low and high irradiation levels on growth and PSII efficiency in *Lemna minor* L.

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Abstract – Plant growth and reproduction depend on light energy that drives photosynthesis. In the present study we compared growth characteristics, photosynthetic pigments content and photosystem II (PSII) performance in *Lemna minor* L. grown in two different irradiation regimes: low light (LL) – 50 $\mu mol_{PHOTONS}\ m^{-2}\ s^{-1}$ and high light (HL) – 500 $\mu mol_{PHOTONS}\ m^{-2}\ s^{-1}$. The main goal was to investigate the photosynthetic regulatory mechanisms that ensure adjustment to different light conditions and integrate these observations with the data on plant multiplication and biomass production. For this purpose, we measured chlorophyll (Chls) and carotenoid (Cars) contents and analyzed the energy fluxes through the PSII by saturation pulse method as well as by Chl *a* transient induction and JIP test. In a comparison of the effect of LL and HL on plant multiplication and fresh biomass, it was shown that the effect on growth was primarily attributed to the biomass reduction in LL while the effect on number of plants was much smaller. Total Chl and Cars contents were decreased in plants exposed to HL which indicated long-term acclimation response to the increased irradiance. Furthermore, the HL plants revealed better capability for the utilization of absorbed light in photosynthesis accompanied by photoprotective adjustment of certain number of PSII reaction centers from active to dissipative mode of functioning. In conclusion, our study showed that duckweed plants had great adjustment potential to different irradiation conditions, which might be of great importance not only under variable light availability but also when simultaneously challenged by some other environmental disturbance (e.g. different pollutants).

Keywords: chlorophyll fluorescence, duckweed, growth, JIP test, light, photosynthesis, photosynthetic pigments, photosystem II.

Introduction

As a source of energy, light is one of the essential environmental factors for plant life in addition to water, mineral nutrients and carbon dioxide. Light intensity, spectral quality, duration and direction of illumination are highly variable, which remarkably influences the dynamics and efficiency of the photosynthesis process and consequently plant growth. To alleviate the effect of rapid light fluctuation on the photosynthetic apparatus and to adjust light availability with the metabolic demands of an organism, regulatory mechanisms of photosynthesis harmonize light capture and its utilization (Roach and Krieger-Liszkay 2019, Vojta et al. 2019).

Photosynthetic pigments – chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Cars) have a crucial role in light energy capturing. They are assembled into light harvesting antenna complexes (LHCs) which deliver excitation energy into the reaction center of photosystems I and II (PSI and PSII) (Morishige and Dreyfuss 1998). Special Chl molecules in the reaction center of PSI and PSII pass excited electrons into electron transport chain in thylakoid membranes

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of chloroplasts. Carotenoids have a dual role in photosynthesis. They are accessory light-harvesting pigments that transfer light energy to Chls, and so expand the wavelength range of absorbed light. They also have a protective role and prevent harmful effects of exposure to high light intensity (Hashimoto et al. 2016). By altering the content of Chls and Cars, plants adjust photosynthesis to variable light conditions. In many studies, Chls and Cars contents and their ratios have been used as an indicator of plant acclimation to light availability (Lichtenthaler and Burkart 1999, Gonçalves et al. 2001, García-Plazaola et al. 2002, Kitajima and Hogan 2003, Walters 2005, Silvestrini et al. 2007).

During absorption of light energy by Chls, and its transfer through LHCs to the reaction centers of PSI and PSII, a small portion of energy is dissipated as heat and re-emitted as light. Energy released as light is called chlorophyll fluorescence (Maxwell and Johnson 2000). Laboratory methods based on induction and analysis of Chl *a* fluorescence, such as the saturation pulse method as well as Chl *a* transient induction and JIP test, have been applied for the evaluation of photosynthetic efficiency in higher plants. These methods have been demonstrated to be very powerful tools for *in vivo* analysis of the plants' photosynthetic performance, since they are inexpensive, nondestructive, potentially repetitive and highly informative concerning the gained data (Maxwell and Johnson 2000, Strasser et al. 2000, Mkandawire et al. 2014).

Duckweeds (family Lemnaceae) are freshwater macrophytes that are broadly distributed in the natural environment and widely used in ecotoxicological and environmental studies. Plant species belonging to this family have a simple structure, small size, rapid growth rate and vegetative reproduction. Easiness of handling in laboratory conditions, high sensitivity and possibility of evaluation of a wide range of endpoints make them suitable organisms for estimation of the effect of various abiotic environmental factors, including light intensity. Among five genera of duckweed family (*Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia*) the species *Lemna minor* L. and *Lemna gibba* L. have been the most frequently used for environmental and laboratory studies (Wang 1990, Mkandawire et al. 2014).

The objective of the present study was to compare growth characteristics, photosynthetic pigment content and PSII performance in duckweed plants grown at two different irradiation regimes: low light (LL) 50 $\mu mol_{PHOTONS}\ m^{-2}\ s^{-1}$ and high light (HL) – 500 $\mu mol_{PHOTONS}\ m^{-2}\ s^{-1}$. Based on the reports on *Lemna minor* plants growing at different irradiation levels (Artetxe et al. 2002, García-Plazaola et al. 2002) as well as on a broad knowledge of photosynthesis in sun and shade grown plants (Mathur et al. 2018) we hypothesized that: 1) duckweed plants exposed to LL would reveal retarded growth and decrease in biomass gain due to limited photosynthetic potential; and 2) HL grown plants would adjust their photosynthetic performance by decreasing the amount of photosynthetic pigments and by modulating the PSII functioning in such a way as to obtain the most efficient photosynthetic performance associated with effective protection from photodamage. Our aim was to analyze the energy fluxes through the PSII with the intention of detecting which part of electron transport is predominantly adjustable to different irradiation levels.

Materials and methods

Plant material and growth condition

Axenic stock cultures of duckweed (*Lemna minor* L.) were maintained on a modified Pirson-Seidel's nutrient solution (Pirson and Seidel 1950) containing (in mmol L^{-1}) KNO_3, 3.95; $CaCl_2 \times 2\ H_2O$, 5.46; KH_2PO_4, 1.47; $MgSO_4 \times 7\ H_2O$, 1.21; sucrose, 29.2; asparagine, 0.66; and (in μmol L^{-1}) $Na_2EDTA \times 2\ H_2O$, 49; Fe-citrate, 20; $MnCl_2 \times 4\ H_2O$, 1.5; H_3BO_3, 8.1. Before sterilization of the medium (20 min at 121 °C and 0.15 MPa) the pH value was adjusted to 4.5 using solution of KOH. Plants were grown under controlled chamber conditions (Vötsch, Industrietechnik GmbH, Germany) at light intensity 70 $\mu mol_{PHOTONS}\ m^{-2}\ s^{-1}$ provided by wide spectrum fluorescent tubes and a light:dark cycle of 14:10 hours. Temperature was 30 °C during the light and 26 °C during the period of darkness. Cultures were subcultured biweekly.

For the experiment, 2–3 healthy duckweed colonies were put into 100 mL Erlenmeyer flasks containing 60 mL of the nutrient medium. Cultures were divided into two groups. The first one was exposed to low light (LL, PPFD = 50 $\mu mol_{PHOTONS} m^{-2}\ s^{-1}$) and the second group to high light (HL, PPFD = 500 $\mu mol_{PHOTONS}\ m^{-2}\ s^{-1}$) at the plant level. Other chamber conditions were the same as described before. Each group of plants was prepared in 15 biological replicates (15 Erlenmeyer flasks) and exposed to LL or HL for 10 days. To minimize differences in light intensity in each group, Erlenmeyer flasks were repositioned during the experiment. The experiment was repeated two times.

Growth assessment

Growth was evaluated by counting fronds daily during 10 days of the experiment and weighing the fresh biomass at the beginning (1st day) and at the end (10th day) of the experiment. For frond number (N), all visible fronds were counted. Fresh biomass (m) was determined after gently drying the plants between layers of paper towel. Growth parameters were calculated as described in Ensley et al. (1994).

Multiplication of plants was expressed as relative growth of frond number (RG_N) and calculated from the equation $RG_N = (N_t - N_0) / N_0$, where N_t is the number of fronds at day t (t = 2 to 10) and N_0 is the number of fronds at the beginning of the experiment (day 1). For fresh biomass (RG_m), the equation $RG_m = (m_{10} - m_0) / m_0$ was used, where m_0 and m_{10} are biomass at the initial (day 1) and the final day (day 10) of the experiment, respectively.

RG_N and RG_m were expressed as means of 15 replicates (± standard deviation, SD).

Photosynthetic pigments analysis

Fresh duckweed plants (about 0.1 g) were harvested from the flasks, quickly rinsed with distilled water, dried with cellulose fibers and weighed. Plant material was homogenized in 100% acetone, extracted by vortexing and centrifuged at 5000 × g for 10 min. Re-extraction of the pellet was done until tissue was discolored. Supernatants were combined and the volume was measured. For each treatment (LL and HL), 15 separate extractions were done. The concentrations of Chl *a*, Chl *b*, Chl (*a*+*b*) and Cars were determined spectrophotometrically (Specord 40, Analytik Jena, Germany) according to Lichtenthaler (1987). Pigment concentration was expressed as mg per g of fresh weight (FW).

Saturation pulse method

The PS II functional capacity was determined by the saturation pulse method (Schreiber et al. 1995). Chlorophyll *a* fluorescence was measured with the use of a pulse-amplitude-modulation fluorimeter (Mini PAM, Walz, Germany). Plants were dark-adapted for 30 minutes before measurement. Chlorophyll *a* fluorescence was measured from the upper face of duckweed fronds placed on filter paper that was continuously moisturized in order to avoid stress by drying of the plants. Minimal (F_0) and maximal (F_m) fluorescence yields were measured using dark-adapted leaves. The steady-state (F) and maximum fluorescence (F_m') were measured upon application of actinic light of 800 μmol m^{-2} s^{-1}. The actinic irradiation was maintained until both, F and F_m' became stable during application of saturation pulses (5000 μmol m^{-2} s^{-1}), applied every 5 minutes. The values of the maximum quantum yield of PS II (F_v/F_m), the effective quantum yield ($\Delta F/F_m$'), the relative electron transport rate (rel. ETR) as well as the nonphotochemical quenching (NPQ) and photochemical quenching coefficient (qP) were calculated according to Maxwell and Johnson (2000). For each treatment (LL and HL) 15 separate measurements were done. All calculated parameters are expressed as relative units (r. u.).

Chlorophyll *a* transient induction and JIP test

The chlorophyll *a* fluorescence induction kinetics was measured using plant efficiency analyzer (Handy-PEA, Hansatech, UK). Plant material was dark-adapted for 30 minutes before measurement. The OJIP transients were recorded from 50 μs (F_0) to 1 s (F_m) by application of the pulse of saturating red light (3200 μmol m^{-2} s^{-1}, peak at 650 nm) on the upper face of duckweed frond. For each treatment (LL and HL) 25 separate measurements were done on plants taken from 15 Erlenmeyer flasks, therefore from each biological replicate 1–2 plants were taken for measurement. Obtained data were used to calculate the following JIP-test parameters: maximum quantum yield of PS II (F_v/F_m), performance index (PI_{ABS}), density of reaction centers on chlorophyll basis (RC/ABS), flux ratio of trapping per dissipation (TR_0/DI_0) and electron transport beyond the primary electron acceptor Q_A^- ($ET_0/(TR_0-ET_0)$), density of active reaction centers (RC/CS), phenomenological dissipation of excess excitation energy per irradiated leaf cross section (DI_0/CS) as well as the absorption (ABS/RC), trapping (TR_0/RC), electron transport (ET_0/RC) and dissipation (DI_0/RC) fluxes per active reaction center (RC), respectively (Strasser et al. 2000). All calculated parameters are expressed as relative units (r. u.).

Statistical analyses

The data obtained were arranged in two groups (LL and HL) and compared with Student's *t*-test. Results are presented as means ± standard deviation. Differences between mean values were considered statistically significant at $P < 0.05$. Statistical analysis was performed using STATISTICA 10.0 (StatSoft Inc., Tulsa, OK, USA) software package.

Results

In this work, plant growth, photosynthetic pigments content and chlorophyll fluorescence-based parameters were measured to determine the effect of two light intensities (LL and HL) on photosynthetic efficiency in duckweed (*L. minor*).

Plant growth

Comparison of relative growth rates (RG_N) of duckweed exposed to two different light intensities (LL or HL) revealed a slightly different growth pattern of these two groups of plants (Fig. 1). At the beginning of the 10-day growth period (the first three days) there was no significant effect of light intensity on frond number. At day 4, plants exposed to LL showed higher RG_N than plants exposed to HL. After this time point, the dynamics of multiplication of both groups of plants was equal till the 8th day of cultivation. Towards the end of the experiment, the growth of plants exposed to

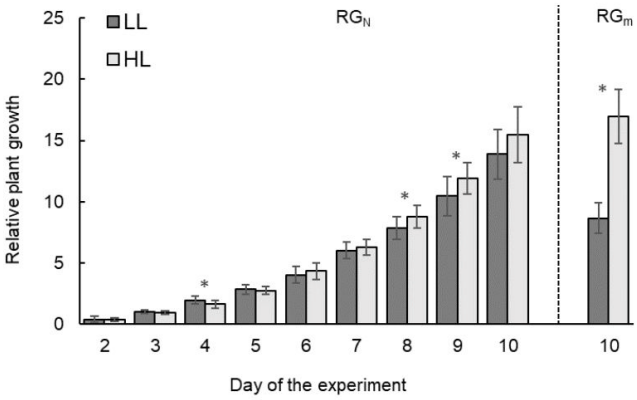

Fig. 1. Relative plant growth (RG) calculated from frond number (RG_N) and fresh biomass (RG_m) of *Lemna minor* cultivated 10 days under low light (LL, 50 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) or high light (HL, 500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) conditions. Plants were counted daily. Fresh biomass was determined at the first and the last day of the experiment. Results, expressed as means of 15 replicates ± SD, were compared with Student's *t*-test and considered significantly different at $P < 0.05$. Columns marked by asterisks indicate a significant difference between groups of plants for each exposure time (2-10 days). SD – standard deviation.

HL was more prominent than in plants exposed to LL. The 10th day of cultivation could also be included into this general conclusion because P value was 0.0508, i. e. it was very close to 0.05.

Fresh biomass, determined at the beginning of the experiment and at day 10, showed significantly higher RG_m value in plants exposed to HL (Fig. 1). Comparison of measured growth parameters (RG_N and RG_m) showed that growth retardation was caused predominantly by biomass reduction while multiplication of plants had a much smaller contribution. In plants exposed to LL, RG_m was 8.67 which is 48.8% decreased value in comparison to 16.95, which was obtained for plants exposed to HL. On the other hand, RG_N was only 10.3% decreased in plants grown under LL in comparison to plants grown under HL (Fig 1).

Photosynthetic pigments

Plants exposed for 10 days to HL (500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) showed significantly decreased Chl *a*, Chl *b* and Cars content in comparison to plants exposed to LL. The content of Chl (*a*+*b*) and Cars in HL plants amounted 0.711 ± 0.070 mg g^{-1} and 0.169 ± 0.024 mg g^{-1}, respectively, which was 39.5% and 21% less than in LL plants (1.175 ± 0.152 mg $g^{-1}$and 0.214 ± 0.022 mg g^{-1}, respectively) (Fig 2A). However, calculated parameters (Chl *a*/Chl *b* and Cars/Chl (*a*+*b*)) showed an opposite pattern and were lower in plants exposed to LL (Fig. 2B).

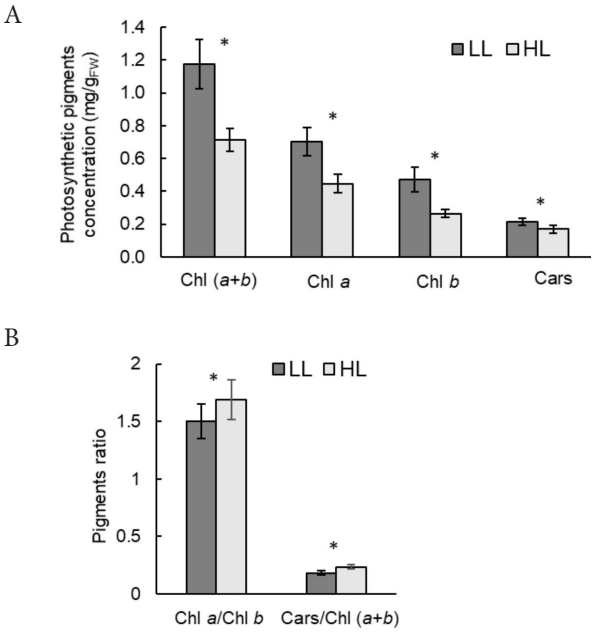

Fig. 2. Concentration (expressed as mg g^{-1} of fresh biomass) of photosynthetic pigments (A) and Chl *a*/Chl *b* ratio and total Cars to total Chl ratio (B) in duckweed (*Lemna minor* L.) after 10 days of cultivation under low light (LL, 50 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) or high light (HL, 500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) conditions. Results, expressed as means of 15 replicates ± SD, were compared with Student's *t*-test and considered significantly different at P < 0.05. Columns marked by asterisks indicate a significant difference between groups of plants exposed to different light conditions. SD – standard deviation.

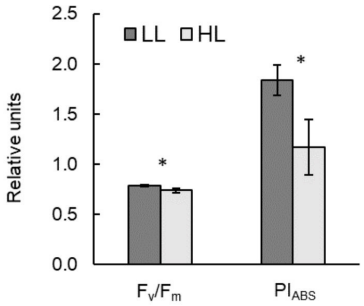

Fig. 3. Maximum quantum yield of photosystem II (F_v/F_m) and performance index (PI_{ABS}) in fronds of *Lemna minor* after 10 days of cultivation under low light (LL, 50 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) or high light (HL, 500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) conditions. Results, expressed as means of 25 replicates ± SD, were compared with Student's *t*-test and considered significantly different at P < 0.05. Columns marked by asterisks indicate a significant difference between groups of plants exposed to different light conditions. SD – standard deviation.

Chlorophyll *a* fluorescence parameters

The value of performance index (PI_{ABS}) in plants exposed to HL (1.170 ± 0.154 r. u.) was about 28% lower than that of plants exposed to LL (1.838 ± 0.275 r. u.). Maximum quantum yield of PS II (F_v/F_m) decreased only about 5.7% in plants exposed to HL (0.742 ± 0.023 r. u.) in comparison to plants exposed to LL (0.787 ± 0.008 r. u.) (Fig. 3).

Analysis of PSII energy fluxes per active reaction center (RC), obtained by chlorophyll *a* induction transient and JIP test, revealed an increase of ABS/RC, TR_0/RC and DI_0/RC in plants grown at HL. However, ET_0/RC values did not change in relation to light intensity and showed similar values at HL and LL (Tab. 1). The density of active reaction centers

Tab. 1. JIP test derived parameters in *Lemna minor* L. plants cultivated 10 days under low light (LL, 50 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) or high light (HL, 500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) conditions. Results, expressed as means of 25 measurements ± SD, were compared by Student's *t*-test and considered significantly different at P < 0.05. SD – standard deviation; ABS/RC, TR_0/RC, ET_0/RC, DI_0/RC – absorption, trapping, electron transport and dissipation fluxes, respectively, per active reaction center (RC); RC/CS – the density of active reaction centers; DI_0/CS – the phenomenological dissipation of excess excitation energy per irradiated leaf cross section; RC/ABS – density of reaction centers on chlorophyll basis, (TR_0/DI_0) – flux ratio of trapping per dissipation; $ET_0/(TR_0\text{-}ET_0)$ – electron transport beyond the primary electron acceptor Q_A^-; P – p-value by Student's *t*-test, NS – not significant.

Parameter	LL-exposed plants	HL-exposed plants	P
ABS/RC	3.028 ± 0.115	3.392 ± 0.228	< 0.01
TR_0/RC	2.383 ± 0.092	2.512 ± 0.139	< 0.01
ET_0/RC	1.429 ± 0.048	1.438 ± 0.062	NS
DI_0/RC	0.646 ± 0.036	0.879 ± 0.121	< 0.01
RC/CS	172.496 ± 23.438	98.360 ± 19.903	< 0.01
DI_0/CS	111.469 ± 16.599	87.223 ± 23.927	< 0.01
RC/ABS	0.331 ± 0.013	0.296 ± 0.020	< 0.01
TR_0/DI_0	3.698 ± 0.177	2.899 ± 0.347	< 0.01
$ET_0/(TR_0\text{-}ET_0)$	1.502 ± 0.068	1.347 ± 0.112	< 0.01

(RC/CS) in HL plants was decreased by 57% in comparison to RC/CS in plants exposed to LL. Also, the value of actual phenomenological dissipation of excess excitation energy as heat per irradiated leaf cross section (DI_0/CS) in plants exposed to HL was 22% lower than in LL plants (Tab. 1).

All parameters that are included in PI_{ABS} calculation (RC/ABS, TR_0/DI_0 and $ET_0/(TR_0 - ET_0)$) revealed increased values in plants grown in LL in comparison to plants grown in HL conditions (Tab. 1).

Chlorophyll *a* fluorescence derived parameters obtained by saturation pulse method under high actinic irradiance level (800 μmol m^{-2} s^{-1}) showed effective quantum yields of PSII ($\Delta F/F_m'$) of 0.228 ± 0.022 r. u. and 0.232 ± 0.026 r. u. in LL and HL plants, respectively, and relETR of 92.933 ± 10.379 and 91.227 ± 8.618 in HL and LL plants, respectively). These two parameters showed no difference between plants exposed to different irradiance levels (Tab. 2).

Considering the values of the photochemical quenching coefficient (qP), when exposed to 800 μmol m^{-2} s^{-1}, HL plants had significantly increased qP values (0.689 ± 0.116 r. u.) in comparison to LL plants (0.584 ± 0.04760 r. u.). The non-photochemical quenching of chlorophyll *a* fluorescence (NPQ) also showed significant difference – HL plants had decreased values (1.461 ± 0.196 r. u.) in comparison to LL plants (1.700 ± 0.246 r. u.).

Tab. 2. Chlorophyll *a* fluorescence parameters obtained by saturation pulse method in *Lemna minor* L. plants cultivated 10 days under low light (LL, 50 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) or high light (HL, 500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) conditions. Plants were exposed to the actinic light of 800 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}. Results, expressed as means of 15 measurements ± SD, were compared by Student's *t*-test and considered significantly different at $P < 0.05$. SD – standard deviation; $\Delta F/F_m'$ – effective quantum yield; rel. ETR – relative electron transport rate; qP – photochemical quenching coefficient; NPQ – nonphotochemical quenching; P – p-value by Student's *t*-test, NS – not significant.

Parameter	LL-exposed plants	HL-exposed plants	P
$\Delta F/F_m'$	0.228 ± 0.022	0.232 ± 0.026	NS
relETR	91.227 ± 8.618	92.933 ± 10.379	NS
qP	0.584 ± 0.047	0.689 ± 0.116	< 0.05
NPQ	1.700 ± 0.246	1.461 ± 0.196	< 0.05

Discussion

Photosynthesis is the basic metabolic process that influences plant growth and biomass production. It is dynamic process, highly dependent on environmental conditions including light as a source of energy (Kaiser et al. 2019). Therefore, the evaluation of plant growth can indicate photosynthesis efficiency under different light conditions. Growing under changing irradiation levels, plants have to adjust their photosynthetic machinery in the way to equilibrate the light capturing with its efficient utilization in photosynthesis. This is especially important in conditions of high irradiation when photoinhibition of photosynthesis might occur, which could have a broad range of detrimental effects on plants, from decreased growth to plant death (Roach and Krieger-Liszkay 2019). During the first seven days of our experiment, LL and HL generally had similar effects on multiplication of plants (expressed as RG_N). An exception was the 4th day, when plants exposed to LL showed more prominent growth (Fig. 1). Since stock cultures of plants were grown at 70 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}, a possible explanation for such observation at this time-point could be stress in plants exposed to HL due to an increase of light intensity to 500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}. In a further experimental period (from the 7th till the 10th day), plants exposed to HL showed enhanced multiplication which could be a result of acclimation to HL and in this time period plants used benefits from higher energy irradiation.

Consideration of the effect of LL and HL on growth parameters of duckweed, RG_m showed a greater difference among two groups of plants (Fig. 1). Higher sensitivity of fresh biomass parameter in comparison to frond number has already been observed by Smith and Kwan (1989) and Naumann et al. (2007). They revealed that duckweeds exposed to metal salts continued to multiply but produced smaller fronds, which resulted in similar frond number but significantly lower biomass. Furthermore, our results are in accordance to data published by Artetxe et al. (2002). In their study *L. minor* acclimated to 500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1} showed remarkably higher biomass increase than plants exposed to 50 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}. Starch could have a significant contribution in total plant biomass. The proportion of starch in duckweed can vary from 3% to 75% of dry weight (Yin et al. 2015), depending on growth conditions, including light intensity.

One of the first characteristics of high light grown or sun-grown plants is lower chlorophyll and carotenoid content than in low light grown or shade plants, which is considered to be a long-term regulation mechanism that controls light absorption capacity (Ruban 2009). Such dynamics of the photosynthetic pigments under high irradiation levels, typically present in land plants, is appreciated as a very efficient adaptive plant mechanism to ever-changing natural environmental conditions (Mlinarić et al. 2016) and presently it is of potential interest for genetic improvement of photosynthesis and plant productivity (Nowicka et al. 2018).

Decreased content of Chl (*a*+*b*) and Cars in plants exposed to HL (Fig. 2) is in accordance with reports on the photosynthetic pigments in *L. minor* plants grown at low and high irradiation levels. Artetxe et al. (2002) measured higher Chl content in plants acclimatized to LL (50 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}) than in those grown at HL (500 $\mu mol_{PHOTONS}$ m^{-2} s^{-1}). For the same light regimes García-Plazaola et al. (2002) determined decreased chlorophyll and total carotenoid content and a higher Cars/Chl (*a*+*b*) ratio in HL grown plants, while the Chl *a*/Chl *b* ratio varied very little between the light regimes. Paolacci et al. (2018) studied the effect of a range of different light intensities on two species of the genus *Lemna* (*L. minor* and *L. minuta*) and found that chlorophyll content decreased with increasing light intensities. However, the Chl *a*/Chl *b* ratio did not change significantly. Biochemical and molecu-

lar mechanisms that control photosynthetic pigment accumulation under different irradiation levels are shown to be closely associated with the accumulation of light-harvesting proteins of PSII (LHCII) (Mathis and Burkey 1989, Sato et al. 2015). It is noticed especially in Chl *b* accumulation (Tanaka and Tanaka 2000). It indicates direct correlation of pigments accumulation with the regulation of the PSII efficiency and PSII driven electron-transport rate. Our results on the Cars content (Fig.2) are in accordance with the investigation done by García-Plazaola et al. (2002) who revealed increased level of total Cars in *L. minor* plants grown under low irradiation. In their research, the most of the total Cars in LL plants consisted of lutein and *β*-carotene.

The revealed decrease of F_v/F_m was less (5.7%) than the decrease in PI_{ABS} (28%) in plants exposed to HL (Fig. 3) indicated that the high irradiation level caused only minor effects to the primary photochemistry of PSII. The primary photochemistry of the PSII is influenced by the absorption and trapping processes. So, we compared the values of the PSII energy fluxes per active reaction center (RC) in plants exposed to LL and HL (Tab. 1). The plants grown in HL revealed increased ABS/RC, TR_0/RC and DI_0/RC, while there was no difference between HL and LL plants for the ET_0/RC value. The ABS/RC parameter is considered as measure for the average PSII antennae size and its increase in plants exposed to HL likely indicates overloading of PSII RC with electrons. This increased the values of dissipation of excess excitation energy per active reaction centers (DI_0/RC) in HL-grown plants and, in turn, decreased the density of their active reaction centers (RC/CS) for 57% in comparison to plants exposed to LL (Tab. 1).

The performance index (PI_{ABS}) is a multi-parametric expression that takes into account the properties of several PSII energy fluxes, such as absorption of light energy, trapping of excitation energy, conversion of excitation energy to the photosynthetic electron transport and the dissipation of excess excitation energy as heat. Therefore, it is considered a very good indicator of overall plant vitality (Tsimilli-Michael et al. 2000, Van Heerden et al. 2007). All parameters that are included in PI_{ABS} calculation (RC/ABS, TR_0/DI_0 and $ET_0/(TR_0 - ET_0)$ revealed increased values in LL-grown plants in comparison to HL-grown plants (Tab. 1). This clearly showed that plants under LL assembled their PSII in such a way as to handle the absorbed and trapped light energy better than HL-grown plants in the given growth conditions.

Further, we investigated several chlorophyll *a* fluorescence derived parameters (relETR, qP and NPQ) with the aim of revealing whether some *in situ* short-term functional differences in photochemical and non-photochemical quenching of chlorophyll *a* fluorescence under high irradiance levels (800 μmol m^{-2} s^{-1}) were developed by different growth conditions (LL and HL). The values of effective quantum yields of PSII ($\Delta F/F_m'$) and PSII driven electron transport rate (relETR) in HL- and LL-grown plants showed no difference (Tab. 2).

The PSII driven relETR is calculated on the tripartite basis of the $\Delta F/F_m'$, photosynthetically active irradiation and the factor that accounts for the energy distribution between PSII and PSI (Schreiber et al 1995). Although relETR under a certain physiological condition corresponds very well (almost linearly) to the CO_2 fixation rate (Krall and Edwards 1992), it cannot be taken unambiguously as a reliable measure for the overall photosynthetic efficiency, as under some other physiological conditions different processes that compete for CO_2 fixation might take place (Maxwell and Johnson 2000). Also, changes in the antennae size and distribution are reported to occur under high irradiance conditions (Ruban 2009) and this might be highly misleading if relETR were taken as an exclusive parameter in photosynthetic capability assessment of plant samples. In order to make more accurate assessment of the absorbed light fraction that is used in photosynthesis, relETR is usually combined with photochemical quenching coefficient (qP), a parameter also obtained by employing the saturation pulse method (Schreiber et al. 1995). When exposed to actinic light of 800 μmol m^{-2} s^{-1} plants grown in HL had increased qP values in comparison to plants grown in LL (Tab. 2). In contrast to the relETR that relies on the absorbed light energy used in PSII photochemistry, the qP indicates the fraction of the open PSII reaction centers (RCs that are capable for photochemical performance, i.e. Q_A reduction) in light adapted plant samples (Baker and Rosenqvist 2004). Accordingly, the qP value is a direct consequence of the photosynthesis saturation by incident irradiance. When plants were challenged by high irradiance level (800 μmol m^{-2} s^{-1}) PSII appeared to be equally downregulated in both LL- and HL-grown plants, which was also reflected as similar values of relETR (Tab. 2). However, increased qP values in HL-grown plants indicated that they were capable of directing a greater fraction of absorbed light into the photosynthesis than LL-grown plants, when exposed to 800 μmol m^{-2} s^{-1}. This can also be related to the almost two-fold increase of fresh weight of HL-grown plants in comparison to the LL-grown plants (Fig. 1). Better photosynthetic performance under high irradiance conditions enable plants to increase their primary metabolism, particularly starch content, which is of great recent interest in biofuel production (Cui and Cheng 2015).

As described by Schreiber at al. (1995) only a fraction of light absorbed by PSII is directed into the photosynthesis due to electron-transport chain (photochemical energy conversion). Another two fractions are harmlessly dissipated as heat due to non-photochemical processes, and the red auto-fluorescence of chlorophyll molecules. Since these three processes are in competition, which portion of the absorbed light will be used to drive photosynthesis depends on several regulatory mechanisms. The major one is non-photochemical quenching of chlorophyll fluorescence (NPQ), which is a measure of plant efficiency in converting excess energy to heat, with the aim of avoiding photo-damage of the PSII RC's (Maxwell and Johnson 2000). Decreased NPQ values in HL plants nicely corroborates their decreased Cars values in comparison to LL plants. It is also in accordance with other fluorescence parameters obtained by the saturation pulse method (Tab. 2) and the data on the heat dissipation per ir-

radiated leaf cross-section (DI_0/CS) obtained by the method of the chlorophyll *a* induction transient and JIP test (Tab. 1). Although investigations of NPQ have been performed for a long time (Bilger and Björkman 1990), its exact molecular mechanism is under constant debate (Niyogi 2000, Nayak et al. 2001, Ruban 2016). What is well known by now is that, basically, NPQ involves conversion of violaxanthin to zeaxanthin through the xanthophyll cycle, which is closely related to structural rearrangements of LHCII complexes that enable xanthophyll conversion and effective excess excitation energy dissipation as heat. However, this is just a piece of the puzzle, since other protective mechanisms such as redistribution of excitation energy between PSII and PSI (Allen 2003) as well as protective role of β-carotene in the PSII RC (Choudhury and Behera 2001) are known to operate in parallel with NPQ.

Based on the presented data it can be concluded that duckweed plants revealed great capability to build their PSII in such a way as to accommodate absorption and trapping processes most effectively at low irradiance growth conditions as well as photochemical energy conversion and NPQ under high irradiance. The described differences in the photosynthetic apparatus assembly and functioning only partially supported our first hypothesis. That is, increased levels of chlorophylls and carotenoids as well as decreased overall photosynthetic performance in LL plants was greatly reflected in biomass production but only slightly in their multiplication in comparison to the HL plants. Our second hypothesis was entirely confirmed, since HL plants adjusted their photosynthetic performance by short-term up-regulation of the part of absorbed light that was directed into the photosynthesis. This was accompanied by photoprotective modification of the fraction of a certain number of RC, from active to dissipative. Furthermore, long-term accommodation of HL plants was evident due to decreased accumulation of photosynthetic pigments. The observed adjustments might have an important role in the photoprotection of duckweed plants growing under different irradiation conditions when challenged by various environmental pollutants. Therefore, the obtained results can serve as a platform for further research into the effects of environmental pollutants on duckweed plants under different irradiation levels.

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