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A simple incubation tank for photosynthesis measurements with six light intensities

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Abstract – The aim of the study was to design and put together a compact, easy-to-assemble and cost-efficient incubation system for aquatic plant photosynthesis measurements. Incubation tank consisting of glass sidewalls and mirror inner walls was constructed. The tank was split into six incubation cells and two water collecting cells. Each incubation cell was built of mirror with reflective side turned into the incubation cells to prevent the self shading of plants and minimized the variance of light intensity within the cell. The wall of each incubation cell facing the source of light was made of 3 mm glass and was covered by light absorbing film. To produce different light intensities single light source (fluorescent tubes) and light absorbing film (3M Scotchtint™ Sun Control RE50NEARL) were chosen. The sidewall of each incubation cell was covered with metal-coated neutral sun control film to produce six distinct light intensities. The different transparency of the sidewalls was achieved by lamination of the chosen film in increasing numbers of layers. The effect of the lamination on optical properties of the film was also studied. The variation of photon flux density within a cell was 3.9%. Continuous use of the system and occasional repetitive measurements of film's transparency showed that it maintained its neutral optical properties over a long period of time.

Key words: Incubation tank / photosynthesis / light intensities / aquatic macrophytes / ecophysiology

Introduction

Irradiance in aquatic environment shows great temporal, spatial and spectral variation (Sand-Jensen, 1989; Björkman and Demmig-Adams, 1994; Kirk, 1996; Wetzel, 2001). Besides the atmospheric attenuation of solar radiation, light in the water column is altered by additional factors like air/water surface reflection, back-scattering, absorption by water and suspended particles, shading effect of aquatic macrophytes and epiphytes (Sand-Jensen, 1989; Kirk, 1996; Van Duin *et al.*, 2001). The great deviation in photon flux density in aquatic environment demands a photosynthetic apparatus with equally great responsiveness.

Photosynthesis is one of the most studied processes of macrophyte physiology (for example: Maberly, 1983; Sand-Jensen and Madsen, 1991; Schwarz and Howard-Williams, 1993; etc.). The sensitivity of photosynthesis to a large amount of abiotic (Adams *et al.*, 1974; Madsen and Sand-Jensen, 1991; Madsen *et al.*, 1996; Short and Neckles, 1999) and biotic factors (Sand-Jensen, 1977; Kirk, 1996; Larcher, 2003) makes it a perfect tool for stress physiological researches, as an indicator process in test studies and environmental risk assessments, etc. (Wang and Freemark, 1995; Nyström *et al.*, 2002; Lambert *et al.*, 2006). The only limitation that stops the spread and usage of plants photosynthesis processes for test procedures is the price and the complexity of equipments used for photosynthesis measurements.

A great variety of non-destructive method of photosynthesis measurements is known. In studies of recent years mostly chlorophyll fluorescence was used for estimation of photosynthetic capacity of a plant (Nyström *et al.*, 2002; Eggert *et al.*, 2003; Cedergreen *et al.*, 2005; Lambert *et al.*, 2006; Shen and Song, 2007). Besides chlorophyll fluorescence oxygen consumption and carbon emission can be utilised in experiments *via* direct measurement with different types of electrodes (Vermaat and Verhagen, 1996; Maberly and Madsen, 1998; Asaeda *et al.*, 2004; Jones, 2005; Shen and Song, 2007; Machova *et al.*, 2008). A number of experiments used special equipments that employ either the O_2 consumption (Kamara and Pflugmacher, 2007 and references) or the CO_2 emission of the photosynthesis (Silva *et al.*, 2005).

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All the above mentioned equipments are factory-built, but substantially could be easily replaced by a cheaper and still effective apparatus made by the scientist itself.

The rate of photosynthesis is mostly affected by irradiance (Adams *et al*., 1974; Madsen and Sand-Jensen, 1991), therefore the most important part of the equipment should be the careful planed light sources. To record the light curve of photosynthesis (photosynthetic response to varying light intensities) a number of light intensities is needed. To produce discrete light intensities for photosynthesis study two alternative ways are possible. The first possibility is the use of multiple artificial light sources to produce each light intensity individually by changing the intensity of the emitted light. Alternatively, there is a possibility of use of a single light source. Different light intensities would be produced by attenuating irradiance of this light source. The former solution can result a spectrally diverse set of light sources and led to deceptive readings of photosynthesis, while the latter produce spectrally identical sources, that differ only in the intensity of light.

In this study we describe a compact, easy-to-assemble and cost-efficient incubation system for measuring aquatic plant photosynthesis. Using this system it is possible to measure the light response curves with six discrete light intensities (and in the dark) on multiple aquatic organisms at the same time. The temperature and the light gradient of the incubation system could be easily modified, which makes the equipment more exploitable.

Materials and methods

In the experiments as source of light metal-halide (A5, Tungsram, Hungary, correlated colour temperature – 4500 K) and fluorescent (F33 Coolwhite, Tungsram, Hungary, correlated colour temperature 4000 K) lamps were used.

For attenuation of emitted light Scotchtint™ Sun Control and Scotchshield™ Ultra Safety and Security Window films (3M, USA) were used. Spectral profile of films was assessed with a spectrophotometer (UV-1601, Shimadzu, Japan).

Spatial distribution of irradiance within the incubation tank was measured with a spherical irradiance meter US-SQS/L sensor (WALZ, Germany) and the data were collected by a LI-COR LI 1400 data logger (LI-COR Biosciences GmbH, Germany).

The control of temperature was maintained by a cooling-heating bath (Neslab RTE-17, Thermo Electron, USA). The temperature within the incubation tank, and each incubation cell was recorded with digital thermometer (Testo mini 525, Testo, UK).

For testing the incubation system on submerged macrophytes *Potamogeton perfoliatus L., Myriophyllum spicatum L., Najas marina L.* and *Ceratophyllum demersum L.* were used. Substrate attached intact plants were collected by hand in the surroundings of the Balaton Limnological Research Institute (46x 54' 46.54" N, 17x 53' 37.53" E) in the best possible condition. At arrival to laboratory no pre-incubation was performed. The 3rd and 4th youngest fully grown leaves (branches of *N. marina*) were detached, thoroughly washed and placed into a Karlsruhe vials, filled with filtered (pore size 0.45 mm) and aerated (95% saturation) lake water. Water filled vials were transferred into the incubator and the temperature of the cooling heating bath was set to actual water temperature of lake Balaton. Mixing was provided by stirring bars and specially made magnetic stirrers (rotation speed – 30 rpm) placed under the incubation tank. Photosynthesis was measured using oxygen electrode (TriOxmatic 300, WTW, Germany) connected to a data logger (Oxi 539 microprocessor precision oxygen meter, WTW, Germany).

Results and discussion

Incubation tank

The incubation tank was designed to fit 6, 235 mm long, 80 mm wide and 110 mm deep incubation cells (Fig. 1 labelled from A to F) and two water collecting cells (80 mm long and 165 mm wide, Fig. 1, a and b). Each incubation cell was considered to take three Karlsruhe vials, while the water collecting cells were planed to simplify the inlet and outlet of water and the regulation of water level within the incubation tank. The inner sidewalls of incubation cells (Fig. 1, solid lines) were made of 3 mm mirror glass with reflective side facing inside each incubation cell. The 4th wall (Fig. 1, dotted line) facing the light source was made of a 3 mm glass. The incubation tank was glued onto the reflective side of 6 mm thick (950 × 180 mm) mirror glass base. The neighbouring incubation cells within rows A-B-C and D-E-F were separated by a 6 mm gap. Each short (80 mm) wall of the incubation cells had a hole and the walls were arranged so that light from the adjacent cells could not get through, but the water would pass through the hole, to the gaps and into the neighbouring cell easily (Fig. 1).

Light source

Metal-halide and fluorescent lamps were used as light sources. A metal-halide lamp produced sufficient amount of light (2000–2500 $\mathrm{mmol \cdot m^{-2} \cdot s^{-1}}$), with optical properties similar to irradiance of the Sun, but with excessive radiation of heat. Illumination of a single incubation cell for 30 min from distance of 10 cm without cooling increased the temperature within the cell from 23.0¡ 0.0 to 47.7¡ 1.5 xC. In contrast, a fluorescent tube produces only a fraction of natural light's irradiance (200–250 $\mathrm{mmol \cdot m^{-2} \cdot s^{-1}}$) with spectral properties that differ from those of the Sun, but since it uses non-thermal radiation processes to produce light, the amount of emitted heat is not significant. The temperature in a single incubation cell after 30 min of illumination with fluorescent

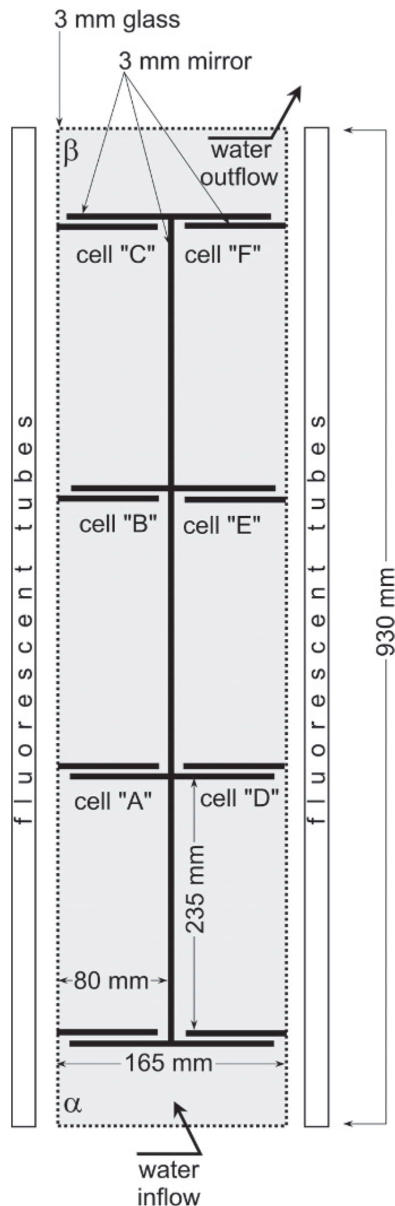

Fig. 1. Top view of the incubation tank showing six incubation (from A to F) and two collecting cells (a and b). The inner sidewalls of each incubation cell were made of 3 mm mirror glass (bold lines) placed with reflective side in. The sidewalls of the incubation tank were made of 3 mm glass (dash line). The whole structure mounted on a 6 mm mirror glass base with reflective side up.

lamp from a distance of 10 cm without cooling increased by 5.4¡ 0.6 xC. To achieve the necessary maximal irradiance for the photosynthesis measurements five 36 W fluorescent lamps were arranged on a very short distance parallel to each other. As a result of this arrangement maximal light intensity of 1200 mmol$\cdot$m$^{-2}\cdot$s^{-1} was reached. In our experiments F33 Coolwhite fluorescent lamps were used due to their relatively uniform radiation in the photosynthetically active radiation (PAR) range (only two significant energy peaks in the spectral power distribution).

To test the thermal capacity of the system we placed 18 Karlsruhe vials (three in each incubation cell) into the incubation tank. The vials and the incubation tank were filled with distilled water. The incubation cells closest to water inflow from the bath were the cell A and D, while the farthest were C and F (Fig. 1). The cooling-heating bath was set to room temperature (22 xC). The temperatures in the Karlsruhe vials were recorded with digital thermometer.

At full water flow of the bath (15 L$\cdot$min^{-1}) the fluorescent lamps didn't increased the temperature significantly. At the end of one hour illumination period the difference between the temperature of the cell "A" and cell "C" was 0.2¡ 0.1 xC (t-test, $t = -0.71$, $P = 0.52$). Contrary, metal-halide lamps increased the temperature significantly, since cell "C" was 1.7¡ 0.4 xC (t-test, $t = -3.78$, $P = 0.02$) warmer than incubation cell "A". The rest of experiments were performed using fluorescent lamps.

Attenuation of light

As light absorbing material three types of Scotchtint^{TM} Sun Control films (NEARL types: medium neutral, light neutral and very light neutral) and four types of Scotchshield^{TM} Ultra Safety and Security Window films (NEAR400 types: very high performance neutral, high performance neutral, medium neutral and light neutral) (3M, USA) were used. Each film was glued to a 10 mm $\times$ 50 mm piece of 3 mm glass. Twenty-four hours for drying were allowed. The spectral profile of the glass attached films was tested on a spectrophotometer.

The majority of tested films (5 out of 7) showed fluctuating spectral profile of light transmittance (Fig. 2). The transmittance curves of all Scotchshield NEAR400 type films showed significant (14–24%) decrease at red (*i.e.* 650–700 nm), green (520–570 nm) and blue (440–450 nm) wavelengths (Fig. 2A). The coefficients of variation (C_V) of transmittance of these films were between 0.2 and 1.3, while the glass and the Scotchtint^{TM} RE50NEARL and RE70NEARL films had more stable spectral profile, with relatively low variance ($C_V < 0.05$). The RE50NEARL and RE70NEARL films cut off 53 and 55% of the incident light (Fig. 2B), thus due its lower transmittance 3M Scotchtint^{TM} Sun Control RE50NEARL film was used in further experiments.

Effect of layering on the spectral properties of the film was also tested by measuring the transparency of a test glass (10 mm $\times$ 50 mm pieces of 3 mm glass) with varying number of attached 3M Scotchtint^{TM} Sun Control RE50NEARL films. In the set we compared the spectral profile of transparency of a not coated piece of glass, with five film coated pieces of test glass. Each film coated glass had a different number of layers (from 1 to 5) stratified. Spectral profiles were recorded with a spectrophotometer. Each added layer of 3M Scotchtint^{TM} Sun Control RE50NEARL film decreased the transparency proportionally ($-$52¡ 6%) nearly all through the PAR spectra (Fig. 3). The only significant deviation was