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Effect of LED Light Color on Growth Rates and β-carotene Amounts of *Tetraselmis chuii* and marine *Chlorella* sp.[*]

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Abstract: *In this study, growth rates and pigments of Tetraselmis chuii and marine Chlorella sp. species were examined in different color LED lamps (yellow LED, white LED, blue-red LED). As a result of experiment, highest cell densities were measured with yellow LED light for Tetraselmis chuii as 412x10^4 cell/ml and marine Chlorella spas715x10^4 cell/ml. Also, highest β-carotene amounts were measured with yellow LED light for Tetraselmis chuii and marine Chlorella sp. as 1.07 µg/ml, 0.25 µg/ml respectively*

Keywords: *Tetraselmis chuii,* marine *Chlorella* sp., light, pigments, β-carotene.
[*] *This article is produced from Pınar PİRİNÇ's PhD thesis.*

Renkli LED Işıkların *Tetraselmis chuii* ve Deniz *Chlorella* sp.'nin Büyüme Hızı ve β-karoten Miktarları Üzerine Etkisi[*]

Öz: *Bu çalışmada, Tetraselmis chuii ve denizel Chlorella sp. türlerinin, farklı renkli LED lambalarında (sarı LED, beyaz LED, mavi-kırmızı LED) kültürü yapılarak büyüme ve pigment oranları araştırılmıştır. Deney sonucunda, en yüksek hücre yoğunlukları, Tetraselmis chuii için 412x10^4 hücre/ml ve denizel Chlorella sp. için 715x10^4 hücre/ml olarak sarı LED ışıkta yapılan kültürlerde ölçülmüştür. En yüksek β-karoten miktarları da yine sarı LED ışıkta yapılan kültürlerde Tetraselmis chuii ve denizel Chlorella sp. için sırasıyla 1.07 µg/ml, 0.25 µg/ml olarak bulunmuştur.*

Anahtar sözcükler: *Tetraselmis chuii,* Denizel *Chlorella* sp., Işık, Pigment, β-karoten.
[*] *Bu makale Pınar PİRİNÇ'in Doktora tezinden üretilmiştir.*

INTRODUCTION

Chemical composition of microalgae and growth rates are affected by environmental conditions such as light, spectral range, spectral quality, light intensity and photoperiod. These parameters are effective on growth, physiological activities and biochemical composition of microalgae (Ben-Amotz and Shaish, 1992; Asulabh et al., 2012). Optimum light source and quality are essential because photosynthetical growth is related with light usage of every single cell and their photosynthesis rate increase linearly with light intensity. However, high intensity of light causes inhibition on photosynthesis. If the increment on the light intensity reaches the saturation level, photoinhibition begins to adversely affect the culture. But light intensity bonded with culture depth (Richmond, 2000). Additionally, low light intensities cause restriction on culture growth. Nowadays, LED light sources replace with the traditional light sources and they are highly advantageous on energy consumption when compared with metal-halide lamps or tubular fluorescent lamps. Also, light-emitting diode lamps (LED lamps) are beneficial with their compact structure and durability (Zhao et al., 2011). Due to all these advantages, use of LED light sources became common on microalgae cultures (Wang et al., 2007).

In this paper, effects of different light sources as a LED lamps (yellow, white, red-blue) and fluorescent lamps on growth and pigment composition of marine *Tetraselmis chuii* and *Chlorella* sp., which are used for feeding rotifer to cultivation of gilthead sea bream and sea bass are studied. Most efficient light source will be determined to use on improve production.

MATERIAL and METHODS

Tetraselmis chuii and Chlorella sp. were obtained from the Ege University, Fisheries Faculty, Aquaculture Department, Turkey. These species were cultured in 500 mL glass flasks used as a working volume and trials were done with three replicate. The species were cultured in enriched seawater (3.5 gL^{-1}NaCl) in F/2 medium (Guillard, 1975) and all solutions were autoclaved at 121 °C for 20 min. The vitamin solution was sterilized by passing through 0.22 µm pore size filter paper (Whatman GFC, Germany). Culture temperatures were arranged with air conditioner at 22±2 °C and the cultures were continuously stirred by air without CO_2 addition.

Cultures were illuminated with different LED light on24:0 h photoperiod (L:D) and fluorescent lamp (886.6 lux) was use as a control group. Irradiance levels of LED light systems were measured as yellow LED strips 543.3 lux, white LED strips 783.3 lux, red-blue LED strips 66.6 lux.

Cell numbers were measured using Neubauer hemocytometer and instantaneous growth rates (µ) were calculated with this equation:

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t - t_0}$$

where N_t is the cell number at time t and N_0 is the cell number at time t_0.

The determination of the total concentration of β-carotene was carried out by measuring the absorbance of the different samples using a Hitachi U-2010 Spectrophotometer (Japan). The absorbance of β-carotene was measured at 468 nm and calculated on the basis of a specific absorption coefficient of 4.5. (Zou and Richmond, 2000).

The dry masses of algal species were determined manually by taking samples from culture flasks. A filter paper (0.45 µm, Whatman GFC, Germany) was utilized to determine the dry biomass concentration, which was previously dried in an oven at 105 °C for an hour and weighed for its initial weight. The filter papers were dried in the lab oven after filtration at 105 °C for about 4 hours. Final weight was determined by cooling the filter paper at room temperature. After and before filtration difference in weights divided by the filtered sample volume provided the dry mass concentration. The data were analyzed using factorial of variance (ANOVA) considering pigment concentrations and light sources. When significant differences were found among the groups, Duncan Test was applied statistically (Özdamar, 2004), using the software SPSS 12.0.

RESULTS

It is known that sun is the best illumination source for photosynthesis. However, light inhibition is unavoidable when the light intensity is too high. For this reason, illumination density becomes significant parameter on microalgae cultivation. When the illumination

source and density change, cell adaptations may take time. For both species, culture periods were continued until 26th day, while adaptation time of *Chlorella* sp. was shorter than *Tetraselmis chuii.*

Different light source adaptation of *Tetraselmis chuii* was long for all trials and the first 8 days was recorded as lag phase. Afterwards, exponential growth phase was continued and stagnation phase was started at the day of 18. *Tetraselmis chuii* culture densities were measured as $34.5x10^4$ cell/ml at the first day also gradual increase was observed until the day 20. Fluorescent lamp, yellow LED, white LED and blue-red LEDs culture densities were reached $401x10^4$, $421x10^4$, $203x10^4$, $123x10^4$ cell/ml, respectively. Highest cell density was observed with yellow LED, while lowest cell density was observed with blue-red color LED strip (Figure 1). Additionally, highest β-carotene amount was measured with yellow LED light (1.07 µg/ml) and lowest β-carotene amount was measured with blue-LED LED light (0.10 µg/ml). Furthermore, highest β-carotene amount per cell was measured as 0,0026 µg/ml with yellow LED light at the day 22 (Figure 2). Statistical difference was determined between different light sources on cell density and β-carotene concentrations according to Duncan test ($P<0.05$).

Marine *Chlorella sp.* culture densities were started as $50x10^4$ cell/ml and were increased gradually until the day 22. Yellow LED, white LED and blue-red LEDs culture densities were reached $715x10^4$, $661x10^4$, $330x10^4$ cell/ml respectively (Figure 3). Highest cell density was observed with yellow LED while lowest cell density was observed with blue-red color LED strip. Highest dry weight amount was obtained with yellow LED light. Highest β-carotene amount was measured as 0.25 µg/ml with yellow LED while lowest β-carotene amount was measured as 0.07 µg/ml with blue-red LEDs at 18th day. Additionally, highest β-carotene amount per cell was measured as 0.0004 µg/ml with yellow LED light (Figure 4). Significant statistical difference was determined between different light sources on cell density and β-carotene levels according to Duncan test ($P<0.05$).

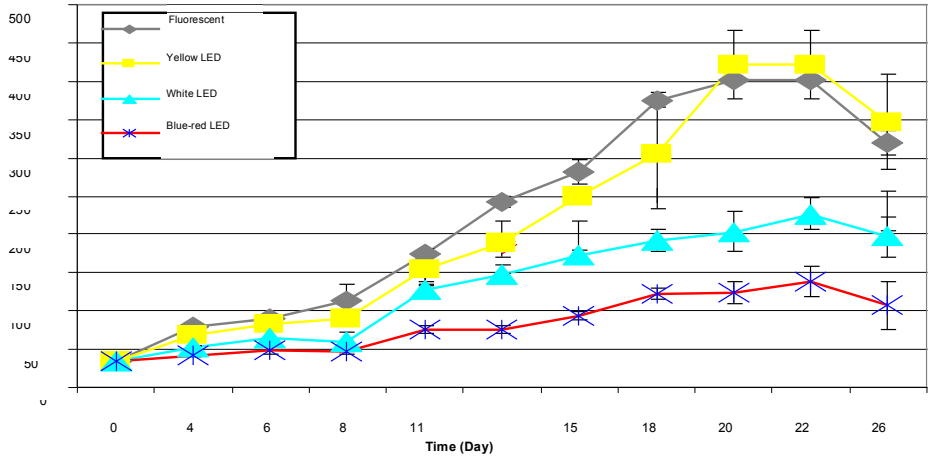

Figure 1. Culture cell density (n x 10^4 cells/ml) of *Tetraselmis chuii* at different light source and color.

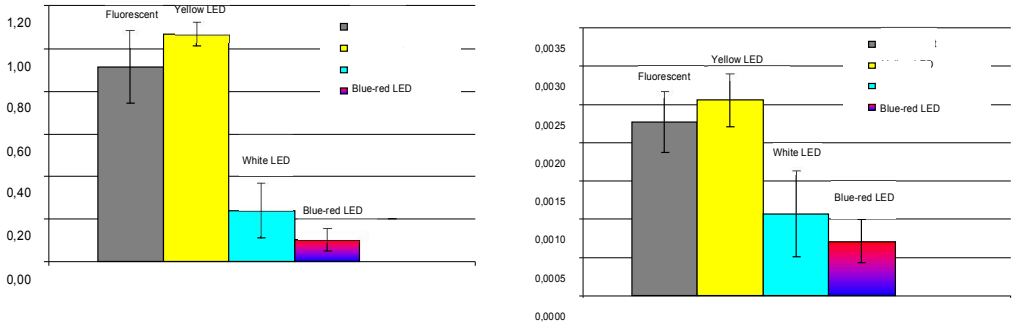

Figure 2. Mean β-carotene of culture (µg/ml of dry mass) and β-carotene of per cell (µg/ml of dry mass) amounts of *Tetraselmis chuii.*

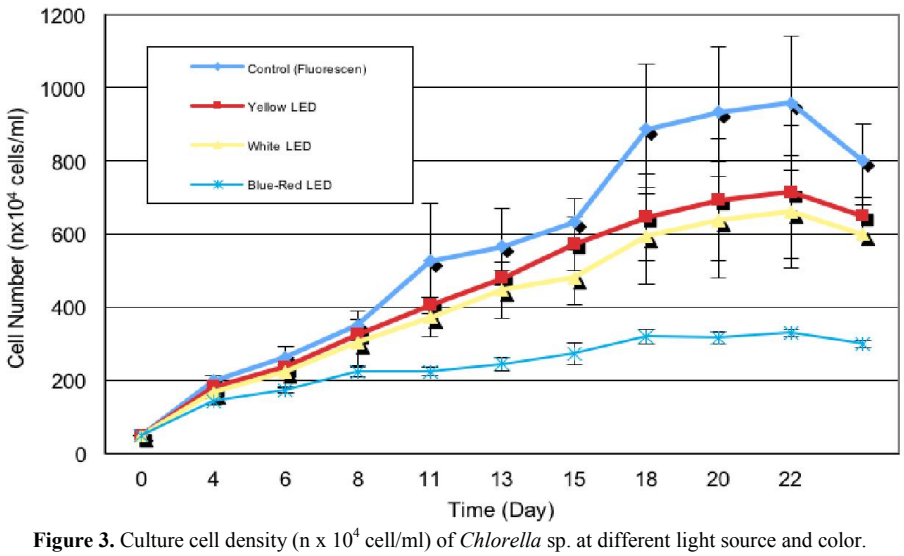

Figure 3. Culture cell density (n x 10^4 cell/ml) of *Chlorella* sp. at different light source and color.

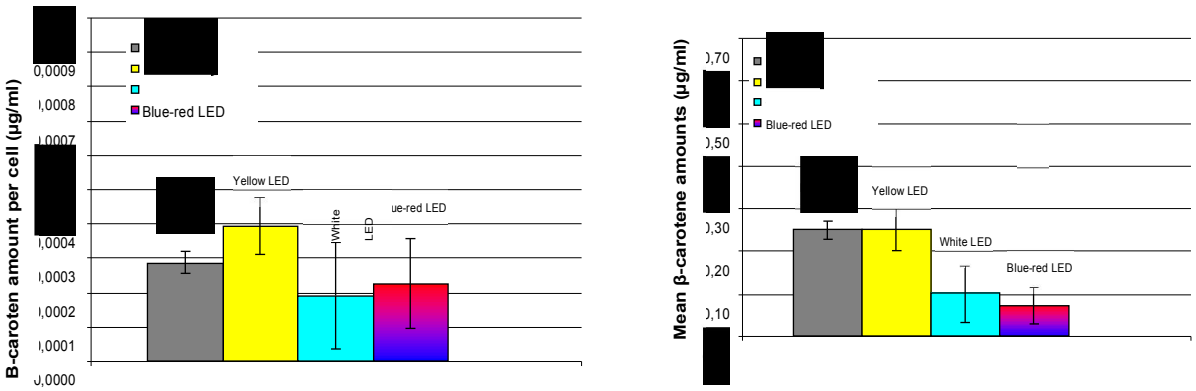

Figure 4. Meanβ-carotene of culture (µg/ml of dry mass) and β-carotene of per cell (µg/ml of dry mass) amounts of *Chlorella sp.*

DISCUSSION and CONCLUSION

The most important parameters which are regulate the algal growth; nutrient quality and quantity, light, pH, turbidity and temperature (Utting, 1985). Optimum culture values differ by species (Coutteau, 1996). Besides, biochemical composition and growth rates of microalgae affected by light, temperature, nutrients and salinity (Ben-Amotz and Shaish, 1992, Asulabh et al., 2012). The main objective of microalgae culture is based on economical values. Especially, low light usage efficiency increases the light source expenses. Therefore, *Tetraselmis chuii* and marine *Chlorella* sp. species and their growth rates, pigment ratios on different light sources was investigated and optimum levels was determined in this study. Environmental conditions, nutrient composition and their concentration alter the microalgal growth and biochemical composition. Culture growth is affected by nutrient composition and its concentration (Brown et al*.,* 1989). F/2 medium, which is appropriate for many species, provides the highest biochemical rates on microalgae culture (Lourenco et al., 2002; Owens et al*.,* 1987; Sukenik et al., 1993; Schneider and Roessler, 1994). Therefore, F/2 medium was chosen for this study. Effectiveness of light type and color on microalgae culture is already known. Wang et al., 2007 showed that in their study the lowest growth rates were obtained with blue LED light while the highest rates was obtained with red LED light. Another study performed by Hultberg et al., (2014), showed that yellow, red and white lights were effective on *Chlorella vulgaris* culture from 6 different wavelengths (Yellow, red, blue, green, white and purple). Red light is important for growth of the *Chlorella vulgaris*. Because, this alga contains very high level of chlorophylls to absorption red light (Fu et al., 2013). In another study, *Chlorella vulgaris* showed highest biomass yield and growth rates with blue light from different light sources such as blue, bright white, green and red (Blair et al., 2014). Red wavelength, yielded favorable results with both *Spirulina platensis* and *Chlorella pyrenoidosa* before (Wang et al., 2007). Another study performed by Abiusi et al., (2014), in the wide spectral range, red LED light was provided increase on EPA when compared with blue, green and white LEDs. Saavedra et al., (1996), obtained higher carotenoid level under the dark red light compared with fluorescent lamp on *Dunaliella salina* culture. Katsuda et al., (2006), investigated blue-red flash lights and their effects on *Haematococcus pluvialis* culture and showed that blue LED flash light increased astaxanthin rate effectively. In this study, experiments were performed with fluorescent lamp, yellow LED, white LED and red-blue LEDs. Red-blue LEDs were provided lowest growth rate on marine *Chlorella* sp.

Another study performed by Das et al., (2011), different wavelengths (red, white, blue and green) and their effect on *Nannochloropsis oculata* culture investigated and from the most effective to less obtained from blue, white, green and red, respectively. Teo et al., (2014), used blue (457 nm), red (660 nm), blue-red, white fluorescent lamp and showed that highest growth rates resulted with blue wavelength on *Nannochloropsis* sp. and *Tetraselmis* sp. cultures.

In this work, *Tetraselmis chuii* and marine *Chlorella* sp. species were cultured under different lights (fluorescent, yellow, white, blue-red LEDs) and highest cell density resulted for *Tetraselmis chuii* and marine *Chlorella* sp. with yellow LED light system. Consequently, short lengt waves like blue requires more energy and causes photoinhibition. Carotenes as a biological antioxidant, have a vital importance because of their protective role on cell stems and tissues from destructive effects (Yanar, 2004). Many researches recommend carotenoids as a protective agent and carotenes such as β-carotene and lutein already used in cancer treatment (Richmond, 2000; Ziegler et al., 1996). In another study, three different light intensities and their relation with chlorophyll a and total carotene values studied and increase in light intensity caused decrease on carotene values determined (Sukenik, 1991). This work showed that highest β-carotene values were obtained for *Tetraselmis chuii* with yellow LED light (1.07 µg/mL). Highest β-carotene values were also obtained for marine *Chlorella* sp. with yellow LED (0.25 µg/ml), while lowest β-carotene values were obtained with blue-red LED systems.

Nowadays, decreasing energy sources and proportionally increase in energy prices effect the product costs. With this reason, experiments on reducing the costs still continues. Considering the data in this study, if we accept basic price without distribution cost for industries 0.12 $/kWh according to Energy Market Regulation Authority of Turkey (EPDK). LED strips (metre) contain 60 LED lamps (4.8W) and which consumes 0.016 $/daily. Lastly, fluorescent lamp (18W) consumes 0.05 $/daily and after consideration of all these calculations, fluorescent lamps consume 5 times more when compared with LED lamps daily.

Consequently, fluorescent lamp and LED light sources differ by energy consumption and its costs. Highest output and lowest production costs are desired targets for microalgae production. LED lights should be chosen because of their low installation costs and long-lasting specifications. Also, culture trials showed that when considering parameters such as culture cell density, β-carotene values and culture illumination costs, LED light systems provide better performance than fluorescent lamps.

REFERENCES

Abiusi F., Sampietro G., Marturano G., Biondi N., Rodolfi L., D'Ottavio M. and Tredici MR., (2014). Growth, photosynthetic efficiency, and biochemical composition of *Tetraselmis suecica* F&M-M33 grown with LEDs of different colors. *Biotechnology and Bioengineering*, **111**(5), 956-964.

Asulabh K.S., Supriya G. and Ramachandra TV., (2012). Effect of Salinity Concentrations on Growth Rate and Lipid Concentration in *Microcystis Sp.*, *Chlorococcum sp.* and *Chaetoceros sp.*, LAKE: National Conference on Conservation and Management of Wetland Ecosystems, School of Environmental Sciences Mahatma Gandhi University, Kottayam, Kerala.

Ben-Amotz A. and Shaish A., (1992). Carotene bio-synthesis. In: Avron, M., Ben-Amotz, A. (Eds.), *Dunaliella*: Physiology, Biochemistry and Biotechnology. CRC Press, Boca Raton, FL, 206-216.

Blair MF., Kokabian B. and Gude VG., (2014). Light and growth medium effect on *Chlorella vulgaris* biomass production. *Journal of Environmental Chemical Engineering* **2**, 665-674.

Brown MR., Jeffrey SW. and Garland CD., (1989). Nutritional aspects of microalgae used in mariculture: a literature review. CSIRO Mar. Lab. Rep., 205, 44.

Coutteau P., (1996). Micro-algae. *In*: Lavens, P. & Sorgeloos, P. (Eds.). Manual on the production and use of live food for aquaculture. FAO Fisheries Technical Paper 361. FAO, Rome, 7-48.

Das P., Lei W., Aziz S. S. and Obbard JP., (2011). Enhanced algae growth in both phototrophic and mixotrophic culture under blue light. *Bioresource Technology,* **102,** 3883-3887.

Fu W., Guðmundsson Ó., Paglia GH., Andresson ÓS., Palsson BØ. and Brynjólfsson S., (2013). Enhancement of carotenoid biosynthesis in the green microalga *Dunaliella salina* with light-emitting diodes and adaptive laboratory evolution. *Appl. Microbiol. Biotechnol.,* **97,** 2395-2403.

Guillard RRL., **(1975)**. Culture of phytoplankton for feeding marine invertebrates in "Culture of Marine Invertebrate Animals.", Smith W.L. and Chanley M.H. (Eds.), Plenum Press, New York, USA, 26-60.

Hultberg M., Jönsson HL., Bergstrand KJ. and Carlsson AS., (2014). Impact of light quality on biomass production and fatty acid content in the microalga *Chlorella vulgaris*. *Bioresource Technology*, **159**, 465-467.

Katsuda T., Lababpour A., Shimahara K. and Katoh S., **(2006).** Astaxanthin Production by *Haematococcus pluvialis* under Illumination with LEDs. *Enzyme and Microbial Technology*, **35**, 81-86.

Lourenco S., Barbarino E., Mancini-Filho J., Schinke K. and Aidar E., **(2002).** Effect of different nitrojen sources on the growth and biochemical profile of 10 marine microalgae in batch culture: An evaluation for aquaculture. *Phycology*, **12**, 249-255.

Owens TG., Gallagher JC. and Alberte RS., **(1987).** Photosynthetic light-harvesting function of viyolaxanthin in *Nannochloropsis* spp. Eustigmatophyceae. *Journal of Phycology*, **23**, 79-85.

Özdamar K., **(2004).** Programmas and Statistical Data Analysis, I. Kaan Press, Eskişehir, 649.

Richmond A., **(2000).** Microalgal biotechnology at the turn of millennium: A Personal View. *Journal of Applied Phycology*, **12**, 441-451.

Saavedra MPS., Jimenez C. and Fiqueroa FL., (1996). Far-red light inhibits growth but promotes carotenoid accumulation in the green microalga *Dunaliella bardawil. Physiol. Plant,* **98**, 419-423.

Schneider J. and Roessler P., **(1994).** Radilabeling studies of lipids and fatty acids in *Nannochloropsis* (Eustigmatophyceae) an oleaginous marine alga. *Journal of Phycology*, **30**, 594-598.

Sukenik A., **(1991).** Ecophysiological considerations in the optimization of eicosapentaenoic acid production by *Nannochloropsis sp.* (Eustigmatophyceae). *Bioresource Technol.,* **35**(3), 263-269.

Sukenik A., Zmora O. and Carmeli Y., (1993). Biochemical quality of marine unicellular algae with special emphasis on lipit composition: II. *Nannochloropsis sp.*. *Aquaculture*, **117**, 313-326.

Teo CL., Atta M., Bukhari A., Taisir M., Yusuf AM. and Idris A., **(2014).** Enhancing growth and lipid production of marine microalgae for biodiesel production via the use of different LED wavelengths. *Bioresource Technology,* **162**, 38-44.

Utting SD., **(1985).** Influance of nitrogen availability on the biochemical composition of three unicelluar marine algae of commercial importance. *Aquacult. Engin.,* **4**, 175-190.

Vonshak A., **(1997).** *Spirulina platensis (Arthrospira)*: physiology, cell biology and biotechnology, Taylor and Francis, London, Great Britain, 213-226.

Wang CY., Fu CC. and Liu YC., (2007). Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. *Journal of Biochemical Engineering,* **37**, 21-25pp.

Yanar Y., Celik M., ve Yanar M., **(**2004). Seosonal changes in total carotenoid contents of wild marine shrimps (*Penaeus semisulcatus* and *Metapenaeus monoceros*) inhabiting the eastern Mediterranean. *Food Chemistry,* **88** (2), 267-269.

Zhao YJ., Hui Z., Chao X., Nie E., Li HJ., He J. and Zheng Z., **(2011).** Efficiency of two stage combinations of subsurface vertical down-flow and up-flow constructed wetland systems for treating variation in influent C/N ratios of domestic wastewater. *Ecol. Eng.,* **37**, 1546-1554.

Ziegler R., Colavito EA., Hartge P., Mc Adams MJ., Schoenberg JB., Mason TJ., and Fraumeni JFJ., **(1996).** Importance of a-carotene, b-carotene and other phytochemicals in the etiology of lung cancer. *J. Natl. Cancer Ins.*, **88**, 612-615.

Zou N., and Richmond A., (2000). Light-path length and population density in photoacclimation. *Journal of Applied Phycology*, **12**, 349-354.

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