

The Effect of Photoacclimation on Photosynthetic Energy Storage Efficiency, Determined by Photoacoustics

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Abstract

Photosynthesis rates in phytoplankton depend on light intensity and its spectral composition, however their relation changes with photoacclimation. During the photoacclimation process algal cells optimize their harvesting and utilization of available light through series of related physical, biophysical, biochemical and physiological changes. These changes result in the ability of phytoplankton to survive under dim light when transported to the depth of the water column and avoid photodynamic damage when exposed to the intense radiation at the surface. Any reduction in the efficiency of light utilization results in decreased rates of photosynthesis rate and slow growth. We present here the study of changes in photosynthetic energy storage efficiency of three phytoplankton species upon photoacclimation to low and high light, as measured by photoacoustics. Our results illustrate the power of photoacoustics as a tool in aquatic ecology and in the physiological research of phytoplankton.

Keywords: Phytoplankton, Photoacclimation, Photosynthesis, Photoacoustics

1. Introduction

Phytoplankton, like all photosynthetic organisms, depend on the capture of light and the transformation of its energy into stored photosynthate, which subsequently fuels all ecosystem activities. For the first step of the photosynthetic process, light has to be intercepted by a variety of light absorbing substances, the photosynthetic pigments. These pigments are associated with proteins, forming light harvesting arrays, or “antennae”, which collect excitation energy and transfer it to the reaction centers of the two photosystems, PSI and PSII that together constitute the photosynthetic unit. These photosynthetic units have a given probability or cross-section for absorbing impinging light photons.

Because of the importance of photoacclimation for phytoplankton which is frequently transported over two orders of magnitude changes in light intensity within hours, this process received considerable attention in the study of aquatic primary production [1-3]. In all the numerous studies of the mechanisms of the photoacclimation process in phytoplankton, a common trend of increase in chlorophyll *a* and in other light harvesting pigments as growth irradiance decreases was observed. Photoacclimation also affects pigment ratios of phyto-

plankton, as light harvesting pigments such as the chlorophylls, phycobilins, fucoxanthin and peridinin increase under low light, whereas photoprotective carotenoids like β carothene, astaxanthin and xanthophyll cycle pigments show the opposite relation with ambient light, increasing whenever exposed to potentially harmful high intense light [4-7]. Most photosynthetic pigments other than chlorophyll *a* are an integral part of the light harvesting antennae. These photosynthetic pigments respond to changes in light intensity in a similar way to that of chlorophyll *a* [8]. Concomitantly, as cellular pigmentation increases in the course of photoacclimation to low light, the cross section of phytoplankton invariably decreases [8,9]. This change in cross section resulting from the increase in mutual shading between cellular light harvesting entities, as their density increases. It happens on all scales, among individual pigment molecules, thylakoids, chloroplasts within cells, and among cells in culture [10]. Upon exposure to high light intensity, the above described trends are reversed [11].

The photoacoustic method, allows the direct determination of the energy storage efficiency of photosynthesis by relating the energy stored by photosynthesis to the total light energy absorbed by the plant material [12-14]. Depending on the efficiency of the photosynthetic sys-

tem, a variable fraction of the absorbed light energy is stored, thereby affecting the heat evolved and the resulting photoacoustic signal.

By exposing the cells to a saturating continuous background light, no storage of any of the pulse energy can take place, whereas in the absence of such light, a maximal fraction of the pulse energy is stored by photosynthesis. Thus the maximal efficiency Φ_{\max} , is determined as the complement of the ratios of the photoacoustic signal, generated by a weak pulse of light in the dark (PA_{dark}), to that obtained under strong continuous light (PA_{light}).

$$\Phi_{\max} = 1 - \text{PA dark} \div \text{PA light} \quad (1)$$

The aim of this work was to examine the photoacoustic method developed by us [15-17] in physiological phytoplankton research. We demonstrate this novel application, by investigating changes in photosynthetic energy storage efficiency occurring in a few phytoplankton species of different taxa, resulting from photoacclimation.

2. Methods and Materials

2.1. Algal Culture

The three species of marine phytoplankton studied were a diatom, *Phaeodactylum tricoratum*, green alga *Nannochloropsis sp.* and golden-brown flagellate *Isochrysis galbana*. All cultures were grown in 250 mL Erlenmeyers flasks containing 200mL enriched artificial seawater medium (Guillard's F/2) [18] at $24^\circ \pm 0.5$ C, under white fluorescent lights at $\sim 10 \mu\text{mol q m}^{-2} \text{s}^{-1}$ (low light (LL)) and $\sim 500 \mu\text{mol q m}^{-2} \text{s}^{-1}$ (high light (HL)).

Cell concentration was measured with a hemacytometer. Chlorophyll content was measured spectrophotometrically in 90% acetone extracts using the equations of Jeffrey and Humphrey [19].

2.2. Photoacoustics

The experimental system is shown schematically in **Figure 1**. The sample was placed in a 16 mm square glass cell (PAC). The laser (L) pulse, after passing through a pair of 1mm wide slits (S) is incident upon the suspension of algae whose pigments absorb part of the laser light. Depending on the experimental conditions, a fraction of the absorbed light pulse is stored in the products of photosynthesis. The remainder of the absorbed light is converted to heat producing an acoustic wave. This is intercepted by a submersible detector (D), containing the ceramic disc. A small portion of the laser pulse is deflected by a beam splitter (BS) and used to trigger the

(Amptek A-250 Preamp and Stanford Research A 560 Amp) photoacoustic signal is recorded. The signal contains a noisy background and later reflections from the walls of the vessel as well as from impedance mismatch within the detector (for details see [17]).

By increasing the continuous background light intensity from zero to saturation of photosynthesis, an increasing fraction of the reaction centers is closed at any time, and a decreasing fraction of the probe laser pulse energy is stored. A corresponding increase in the fraction of the pulse energy is converted to heat, which is sensed by the photoacoustic detector. From these detector responses the photosynthetic energy-storage versus background light-intensity relationship was obtained [15-17].

3. Results and Discussion

In these experiments the photoacclimation of three algal species *Phaeodactylum tricoratum*, *Nannochloropsis sp.* and *Isochrysis galbana* to low and high photon irradiances was examined. In general, photoacclimation to low light results in increased cellular absorption due to high concentration of light-harvesting pigments. In the numerous studies on the mechanism of photoacclimation in phytoplankton, a common trend was reported of increase in chlorophyll as growth irradiance decreases (Falkowski, 1980; 1984; Dubinsky et al. 1986; 1995, Ritz, et al. 2000). As seen in **Figure 2** and **Figure 3** all three species showed a difference in cellular chlorophyll content by $\sim 56\%$ in *Isochrysis galbana*, 38% and 35% in *Phaeodactylum tricoratum* and *Nannochloropsis sp.*, respectively.

The ratios of cellular chlorophyll under the low and high light were 1.415 for *Phaeodactylum tricoratum*, 1.398 for *Nannochloropsis sp.* and 1.984 for *Isochrysis*

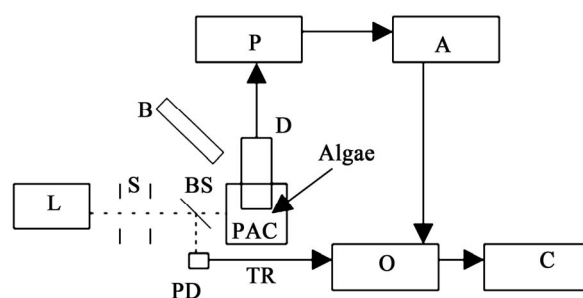


Figure 1. Schematic of the photoacoustic setup: L: laser (Minilite Q Switched Nd :YAG, 532 nm); S: beam shaping slits, BS: beam splitter; PAC: photoacoustic cell with suspension of algae; D: stainless-steel photoacoustic detector, contained a 10-mm diameter resonating ceramic disc (BM 500, Sensor, Ontario, Canada); P: low-noise preamplifier (Amptek A-250); A: low noise amplifier (SRS 560); PD: photodiode; TR: trigger signal, B: background light source, quartz-halogen illuminator (Cole Parmer 4971); O: oscilloscope (Tektronix TDS 430A); C: computer.

galbana. In addition to the changes in cellular chlorophyll most other plant pigments also respond to ambient irradiance [4,23]. All light-harvesting pigments increase under low light. These include the carotenoids fucoxanthin and peridinin, in addition to all chlorophylls, phycoerythrin and phycocyanin [4].

These results are similar to the data cited in the literature, where cellular chlorophyll content increased under low light (LL) conditions and reduced under high light (HL) (Table 1)

The decrease of chlorophyll concentration in HL growth conditions resulted in a parallel reduce in photosynthetic energy storage efficiency **Figure 4**.

We determined the photosynthetic energy storage under different ambient irradiance levels, resulting in an energy storage curve (**Figure 5**). This relationship is similar to the photosynthesis versus irradiance, (P vs I) curve obtained by the tedious standard measurements of ^{14}C fixation and oxygen evolution [24-26] or the indirect results from measurement of variable fluorescence [27].

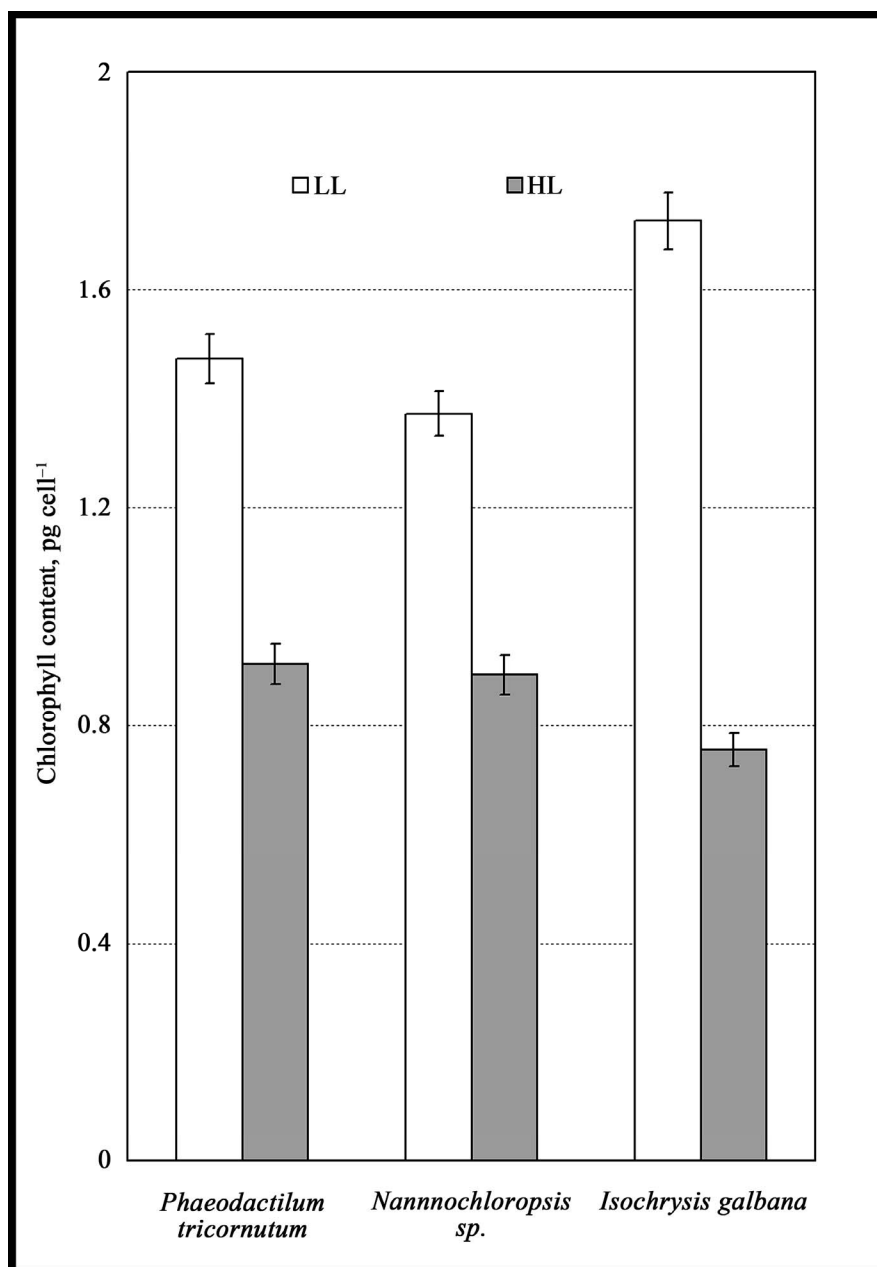


Figure 2. The effect of photoacclimation to high light ($500 \mu\text{mol}\cdot\text{q}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and low light ($10 \mu\text{mol}\cdot\text{q}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) on cellular chlorophyll content for three algae.

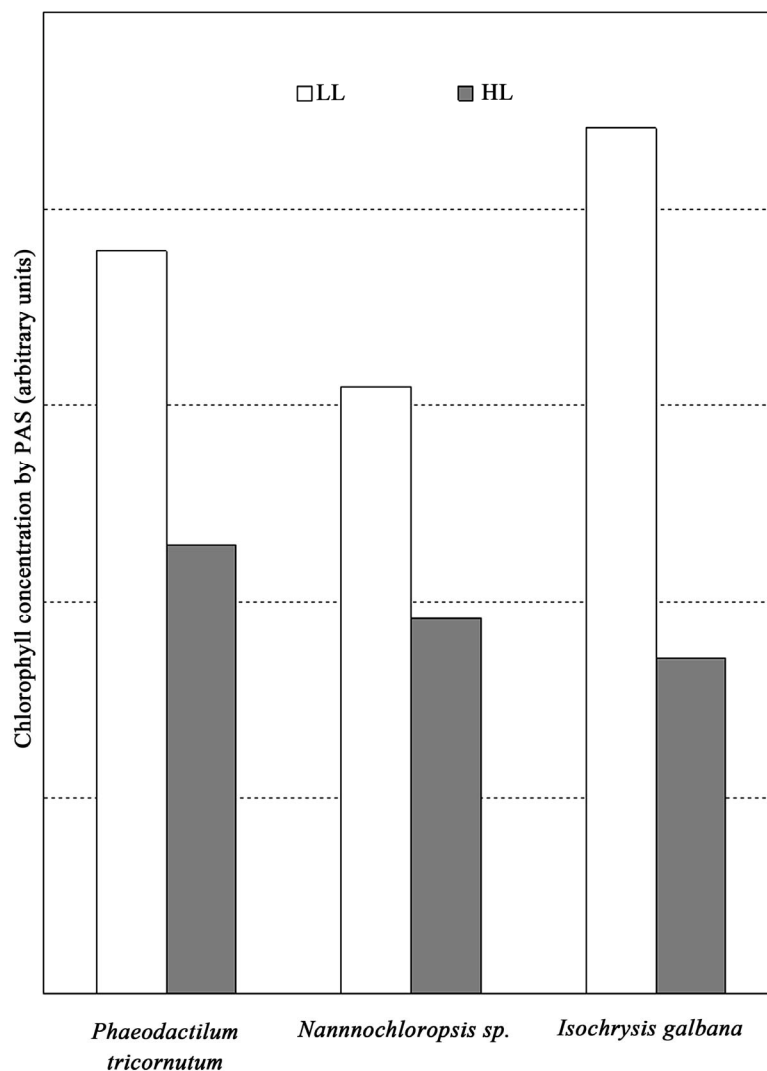


Figure 3. The effect of photoacclimation to high light and low light on chlorophyll concentration measured by photoacoustics.

Table 1. The LL/HL chlorophyll ratios for different phytoplankton species cited in literature.

Algae	Ratio	Reference
<i>Phaeodactylum tricornutum</i>	1.415	Present study
<i>Nannochloropsis sp.</i>	1.398	Present study
<i>Isochrysis galbana</i>	1.984	Present study
<i>Phaeodactylum tricornutum</i>	1.592	Present study by photoacoustics
<i>Nannochloropsis sp.</i>	1.020	Present study by photoacoustics
<i>Isochrysis galbana</i>	2.501	Present study by photoacoustics
<i>Thalassiosira weissflogii</i>	2.486	Dubinsky <i>et al.</i> 1986
<i>Isochrysis galbana</i>	2.130	Dubinsky <i>et al.</i> 1986
<i>Prorocentrum micans</i>	2.351	Dubinsky <i>et al.</i> 1986
<i>Symbiodinium microadriaticum</i>	1.921	Iglesias Prieto and Trench, 1994
<i>Isochrysis galbana</i>	2.076	Herzig and Dubinsky, 1992

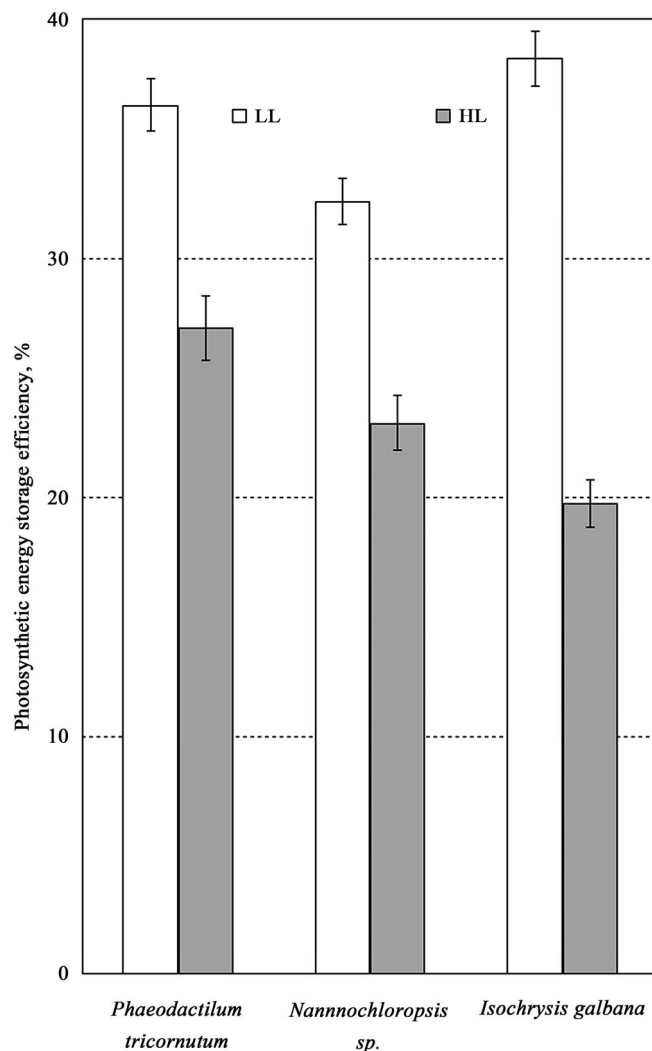


Figure 4. The effect of photoacclimation to high light and low light on photosynthetic energy storage efficiency for three algae.

Figure 5 shows that the photosynthetic energy storage efficiency *Isochrysis galbana* at the initial time of experiments resembles the usual P vs I curves, where photosynthesis increases with light intensity up to the onset of light saturation. Thus, neither the initial slope, α , nor the light saturation parameter E_k , change. These photosynthetic parameters relate to the efficiency of light harvesting by the pigments which seems to remain unaffected. The effect of photoacclimation to high light is however pronounced in the depression of the light saturated rate of photosynthesis P_{max} . After the exposure to HL, all samples shows decrease in photosynthetic energy storage efficiency by 48% in *Isochrysis galbana*, 29% and 26% in *Phaeodactylum tricornutum* and *Nannochloropsis sp.*, respectively.

The results of this study are similar to those carried out in many previous studies [8,21,22,28]. However, these studies used a variety of measures of the photo-

synthetic activity and are thus difficult to compare directly. Many also involved difficult and labor intensive measurements. In contrast, our measurements were all carried out with the same methodology that is simple, quick and direct. The complete measure of activity requires only one minute. Most important, the activity measured by the photoacoustic method is the absolute thermodynamic efficiency of photosynthesis. Therefore, while there is ample evidence that under acclimation to high light there is a universal decrease in cellular pigmentation, photoacoustic reveals what fraction of the absorbed light is dissipated as heat. Indeed, under high light acclimation there is an increase in photoprotective pigments that do not make the light they absorb available for photochemistry but rather dissipate it as heat. Such are some of the carotenoids like β carotene and astaxanthine as well as the pigments involved in the xanthophyll cycle.

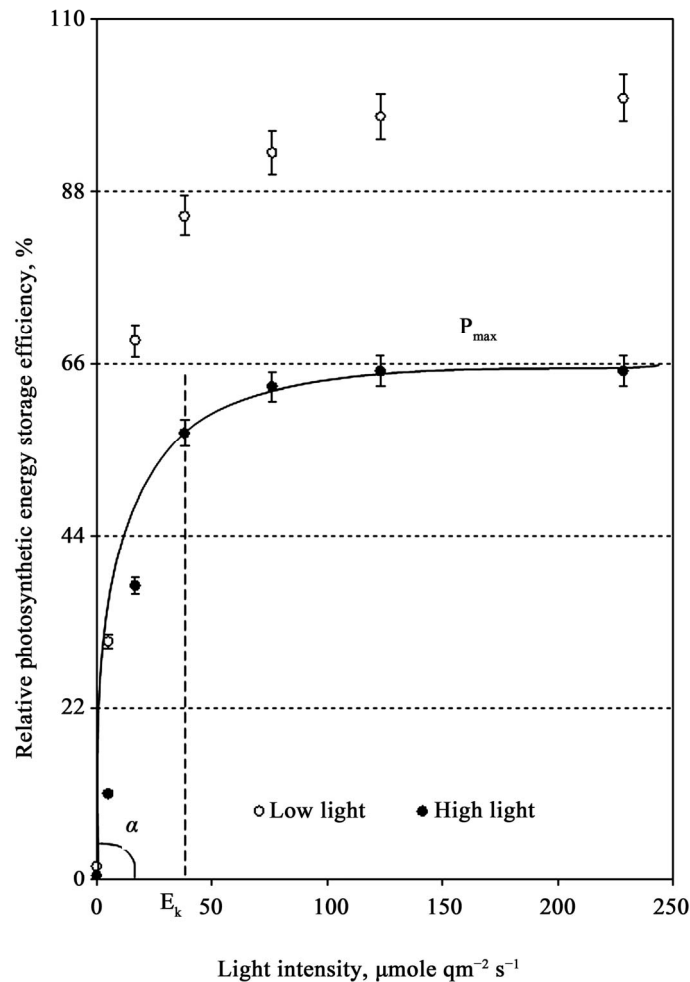


Figure 5. The effect of photoacclimation to high light and low light on relative photosynthetic energy storage efficiency of *Isochrysis galbana*. In order to standardize our results, we converted the absolute energy storage efficiencies to relative ones, setting the maximal storage in low light conditions as 100% photosynthetic energy storage efficiency.

Furthermore, reliable estimates of the quantum yields of oceanic phytoplankton are essential parameters for any modeling of global primary productivity, based on interpretation of satellite images. In summary it is both of considerable scientific interest, and of great applied importance for fisheries and for environmental protection to measure changes over time, as well as over regional variation, of the efficiency of photosynthetic energy storage.

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