Chapter 11

Photoacoustics — A Novel Tool for the Study of Aquatic Photosynthesis

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Photoacoustics

The photoacoustic method allows direct determination of the energy-storage efficiency of photosynthesis by relating the energy stored by it to the total light energy absorbed by the plant material (Canaani et al., 1988; Malkin & Cahen, 1979; Malkin et al., 1990). These authors applied the photoacoustic method to leaves in the gas phase, where brief pulses caused concomitant pulses of oxygen that caused a pressure transient detected by a microphone. This method is based on the conversion of absorbed light to heat. Depending on the efficiency of the photosynthetic system, a variable fraction of the absorbed light energy is stored, thereby affecting the heat evolved and the resulting photoacoustic signal. The higher the photosynthetic efficiency, the greater will be the difference between the stored energy with and without ongoing photosynthesis (Cha & Mauzerall, 1992). These authors collected microalgal cells onto a filter and studied them by an approach similar to that previously used with leaves. In both cases, the oxygen signal is combined with that of thermal expansion resulting from conversion of the fraction of the light energy in the pulse that is not stored by photochemistry.

In the case of liquid algal cultures, there is no signal due to photosynthetic oxygen evolution as gas; hence, the signal detectable by an immersed microphone is proportional to the heat generated by a laser pulse. The light absorbed by the photosynthetic pigments in the algal cells is, in part, stored by photochemistry as products of photosynthesis, while the remainder is converted to heat, causing an expansion of the culture medium. This expansion causes a pressure wave that propagates through the culture and is sensed by the hydrophone. By exposing the cells to continuous saturating 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 photosynthetic storage efficiency, PSmax, is determined from the difference between the signal obtained from a weak laser pulse under strong, continuous illumination (PAsat) and that obtained in the dark (PAdark). The above is then divided by PAsat.

$$PS\ max = \left(PAsat - PAdark\right)/PAsat \tag{1}$$

[For development of equations, see Cha and Mauzerall (1992)].

The experimental setup is shown schematically in Figure 1. The sample was placed in a sample cell. The beam of the brief laser pulse (5ns) is incident upon the suspension of algae, whose pigments absorb part of the laser light (Fig. 2). Depending on the experimental conditions, a variable fraction of the absorbed light pulse is stored in the products of photosynthesis. The remainder of the absorbed light is converted to heat, which causes a transient expansion of the surrounding water, producing an acoustic wave. This is intercepted by a submerged microphone containing a pressure-sensing ceramic disc.

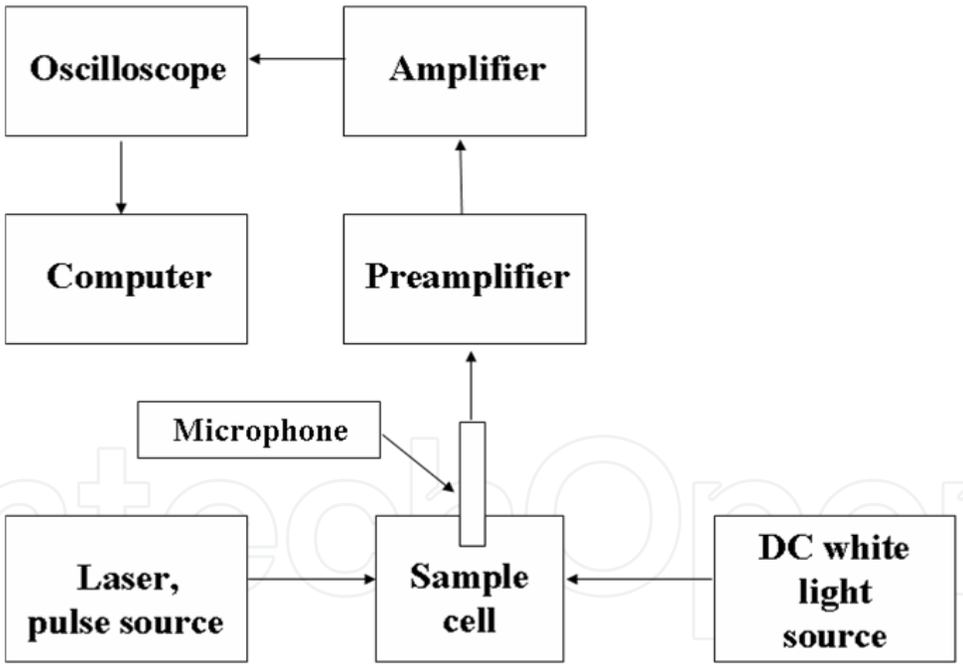

Figure 1. The photoacoustic setup.

A small portion of the laser pulse is used to trigger the Tektronix TDS 430A oscilloscope, where the amplified (Amptek A-250 Preamp and Stanford Research 560 Amp) photoacoustic signal is recorded (computer). An amount of 10 μs of the time scale was found by us as the optimal duration for quantification of the signal, beyond which the signal to noise ratio deteriorates. This time frame allowed us to fire the laser at 10 hz, thus averaging 128-256 pulses.

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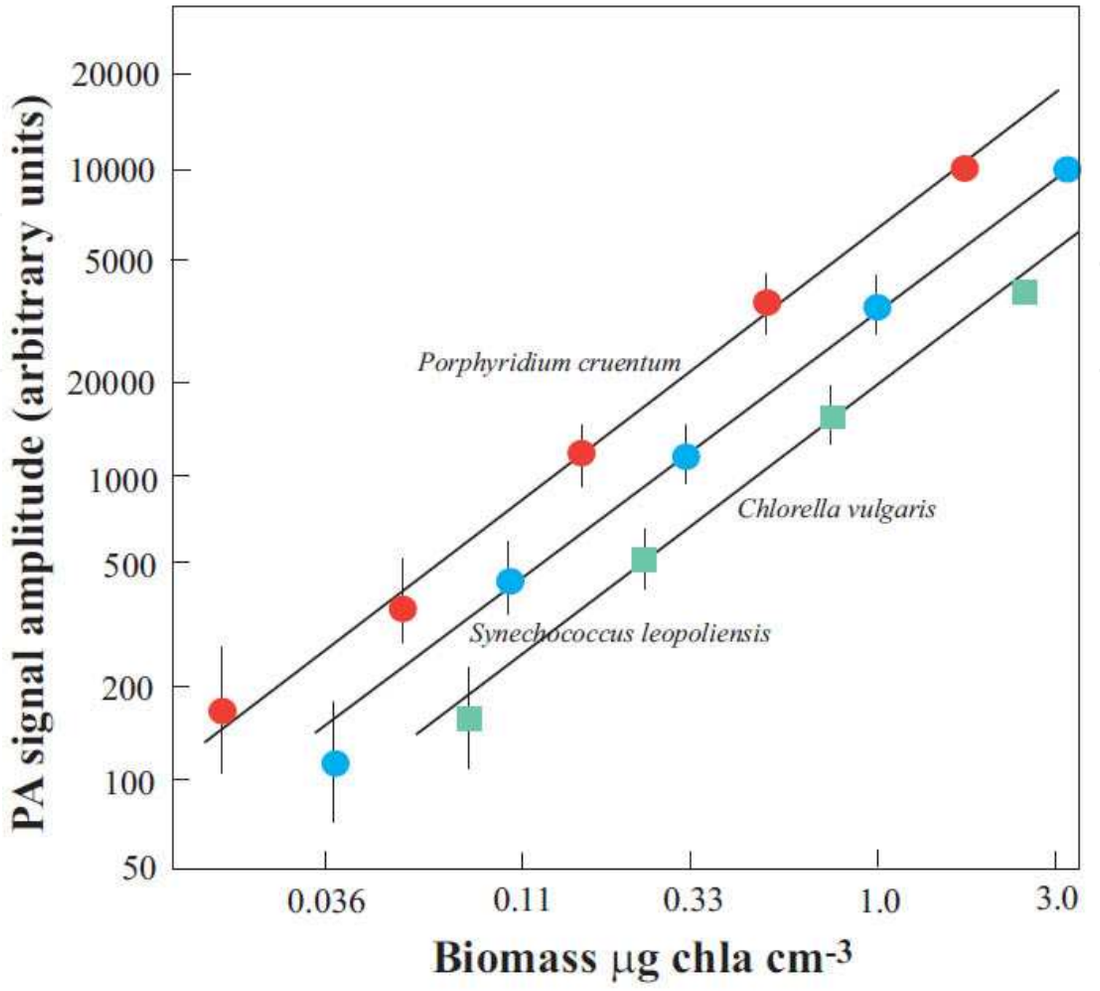

Figure 2. The photoacoustic signal vs Chl a content in 3-fold serially diluted laboratory cultures of three organisms (log_{10} scale: red circles – *Porphyridium cruentum*; blue circles – *Synechococcus leopoliensis*; green circles – *Chlorella vulgaris*. For biomass determination, the light pulse was 430 μJ at 532 nm [according to Mauzerall et al. (1998)]. The laser used was at 532 nm.

2. Quantification of biomass

Biomass detection by photoacoustics is based on the proportionality of the absorbed light to the amount of pigment (Dubinsky et al., 1998; Mauzerall et al., 1998). At high energies, the pulse saturates photosynthesis, and the photosynthetically stored energy becomes a negligible fraction of the absorbed energy. Under these conditions, the photoacoustic signal was proportional to the concentration of chlorophyll over the range of 14 mg to 8.5 μg chl a m^{-3} (Fig. 2) (Dubinsky et al., 1998; Mauzerall et al., 1998). Figure 3 shows the photoacoustic signal of a *Porphyridium cruentum* suspension and the same cells diluted 1:1 with medium (Mauzerall et al., 1998). The advantages of photoacoustics are the strict and exclusive proportionality to the light absorbed by the sample and the ease of obtaining photosynthetic efficiency.

3. Energy storage in photosynthesis

In a photosynthetic system at a given constant light intensity, a fraction of the reaction centers is closed at any time and only part of the light energy is stored (Dubinsky et al., 1998; Mauzerall et al., 1998). Figure 4 shows the photoacoustic signal in the dark (broken line) and saturating light conditions in a suspension of *Porphyridium cruentum*. With no background light ("in the dark"), all reactions are open and the very weak probe pulse causes no saturation, resulting in maximal photosynthesis and minimal heat release (Fig. 4) (Mauzerall et al., 1998).

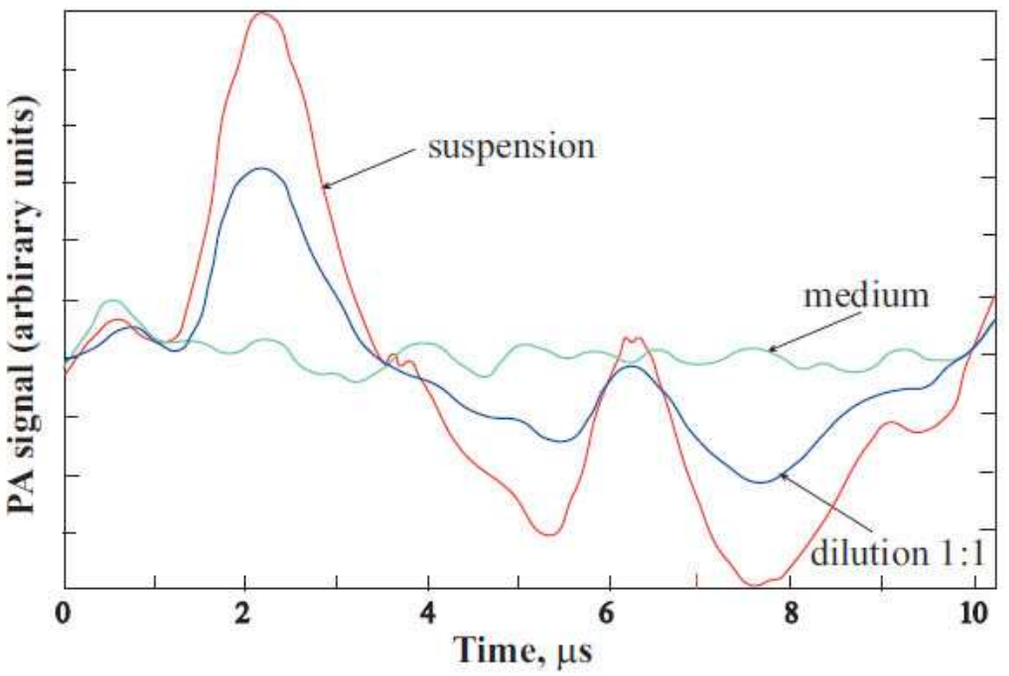

Figure 3. Photoacoustic-signal dependence on concentration of *Phorphyridium cruentum* in suspension. Dotted line is the signal from the (non-absorbing) medium. Red line is for ~0.5 μg/cm^3 Chl *a* and purple is for 1:1 dilution of that solution [according to Mauzerall et al. (1998)].

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 in converted into heat and sensed by the photoacoustic detector. When all reaction centers are saturated, all the probe pulse energy is converted into heat (Fig. 4).

4. Demonstration of applications

We were able to follow the effects of the key environmental parameter, nutrient status, on the photosynthetic activity of phytoplankton and macroalgae. The nutrients examined were iron (Pinchasov et al., 2005), nitrogen, and phosphorus (Pinchasov-Grinblat et al., 2012).

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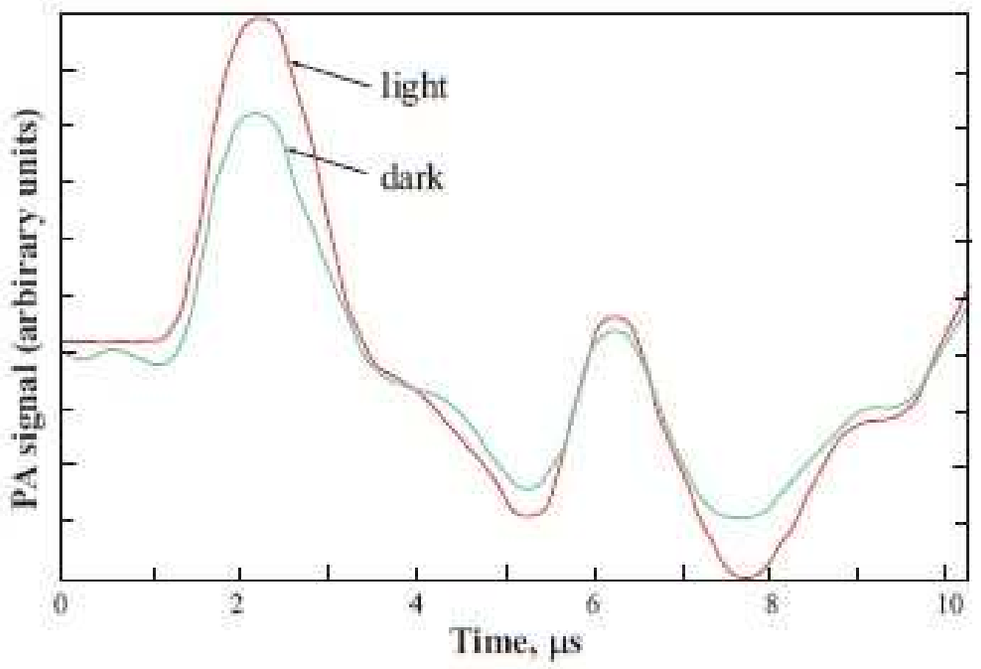

Figure 4. Photoacoustic signal from a *Phorphyridium cruentum* suspension with and without saturating background light (Mauzerall et al., 1998). The area between the curves is proportional to the fraction of the pulse energy stored by photosynthesis.

5. Iron limitation

Three algal species, the diatom *Phaeodactylum tricornutum*, a green alga *Nannochloropsis* sp., and the golden-brown flagellate *Isochrysis galbana*, were cultured in iron-replete media (artificial seawater medium Guillard's F/2) and grown at 24 ºC under white fluorescent lights at ~220 μE m^{-2} s^{-1} PAR. These samples were subsequently transferred to the experimental media containing 0.00, 0.03, 0.09, 0.18, and 0.6 mg L^{-1} iron.

Each culture was diluted in the corresponding medium to chlorophyll *a* concentrations of 5.65 0.1 ± μg ml^{-1} in order to obtain similar absorptivity (Pinchasov et al., 2005).

The photoacoustic experiments were conducted after two weeks in these media. As the iron was progressively depleted, the ability of the three species to store energy decreased (Fig. 5). As seen in Figure 5, all three algal species showed a sharp decrease in efficiency.

6. Photoacclimation

Three species of marine phytoplankton, *Phaeodactylum tricornutum*, *Nannochloropsis* sp., and *Isochrysis galbana*, were studied. All cultures were grown in 250-mL Erlenmeyer flasks containing 200 mL enriched artificial seawater medium (Guillard's F/2) at 24 ± 0.5 °C, under white fluorescent lights at ~10 μmol q m^{-2} s^{-1} [low light (LL)] and ~500 μmol q m^{-2} s^{-1} [high light (HL)].

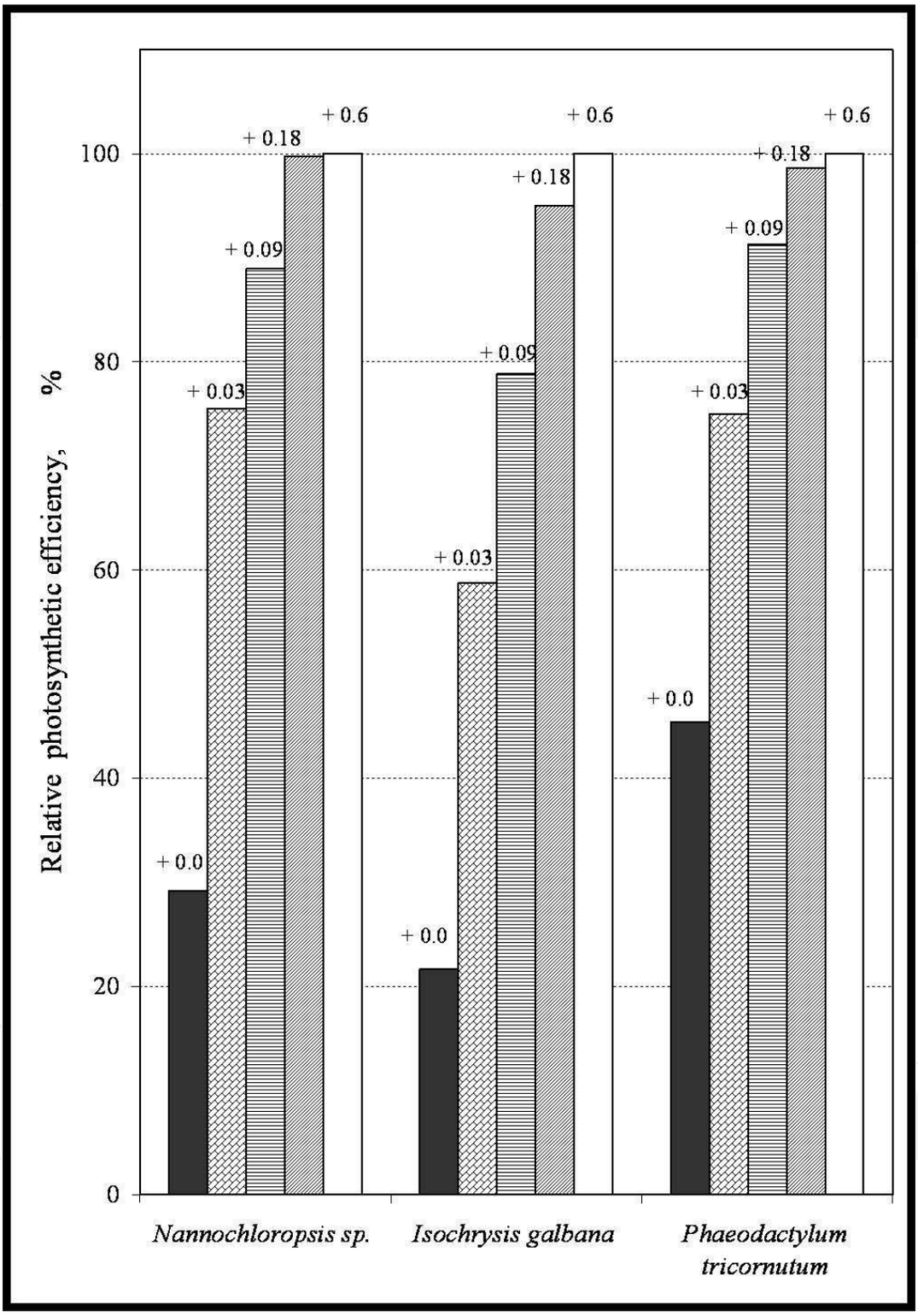

Figure 5. The effect of different iron concentrations on the relative photosynthetic efficiency in the three algae, *Nannochloropsis* sp., *Phaeodactylum tricornutum*, and *Isocrysis galbana*. For each species, the photosynthetic efficiency of the nutrient-replete control was taken as 100%. Controls (clear columns) were grown in iron-replete media contains 0.6 mgL^{-1}. The iron concentration in the iron-limited cultures (hatched columns) was 0 mg L^{-1}, 0.03 mg L^{-1}, 0.09 mg L^{-1}, and 0.18 mg L^{-1} (Pinchasov et al., 2005).

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In these experiments, the photoacclimation of the three algal species, *Phaeodactylum tricornutum*, *Nannochloropsis* sp., and *Isochrysis galbana*, to low and high photon irradiances, was examined (Pinchasov-Grinblat et al., 2011). In general, photoacclimation to low light, results in increased cellular absorption due to a high concentration of light-harvesting pigments. In the numerous studies on the mechanism of photoacclimation in phytoplankton, a common trend of increase in chlorophyll and in thylakoid area as growth irradiance decreases (Dubinsky et al., 1986; Falkowski et al., 1986), was found. In addition to the changes in cellular chlorophyll, most other plant pigments also respond to ambient irradiance. All light-harvesting pigments increase under low light. These include the carotenoids fucoxantin and peridinin, in addition to all chlorophylls, phycoerythrin, and phycocyanin. The decrease of chlorophyll concentration under high-light growth conditions resulted in a parallel reduction in photosynthetic energy storage efficiency, as seen in Figure 6. All three species showed a decrease in efficiency for high-light acclimated algae compared to low-light grown conspecifics: by ~53% in *Isochrysis galbana*, and 33% and 31% in *Phaeodactylum tricornutum* and *Nannochloropsis* sp., respectively.

7. Lead exposure

In our experiments, the exposure of the cyanobacterium *S. leopoliensis* to different concentrations of lead resulted in major changes in photosynthesis (Pinchasov et al., 2006). Figure 7 shows the changes in photosynthetic efficiency following lead application. The reduction of photosynthesis reached ~50% and ~80% with 25 ppm and 200 ppm, respectively. Most of the decrease seen after the first 24 h already took place in the first 40 min.

With an increasing lead concentration and duration of exposure, the inhibition of photosynthesis increases. Since the photoacoustic method yields photosynthetic energy storage efficiency, the results are independent of chlorophyll concentration, which means that the observed decrease in efficiency is not due to the death of a fraction of the population, but rather due to the impairment of photosynthetic function in all cells, possibly due to the progressive inactivation of an increasing fraction of the photosynthetic units.

8. The effect of nutrient enrichment on seaweeds

Samples of the macroalga *Ulva rigida* were collected from the intertidal abrasion platforms in the Israeli Mediterranean during spring 2010. All samples were kept at 22 ± 0.1 ^{o}C in 100 mL Erlenmeyer flasks during 192 h under continuous irradiance at ~200 ± 5.0 μI m^{-2} s^{-1}.

The samples were exposed to 3 treatments: nitrogen (added as $NaNO_3$ at a concentration of 3.25 gL^{-1}), phosphorus (added as NaH_2PO_4 at a concentration of 0.025 gL^{-1}), and nitrogen and phosphorus together. Controls were kept in unenriched seawater.

Nutrient limitation, on the one hand, and anthropogenic eutrophication, on the other, are among the most important factors determining the overall ecological status of water bodies.

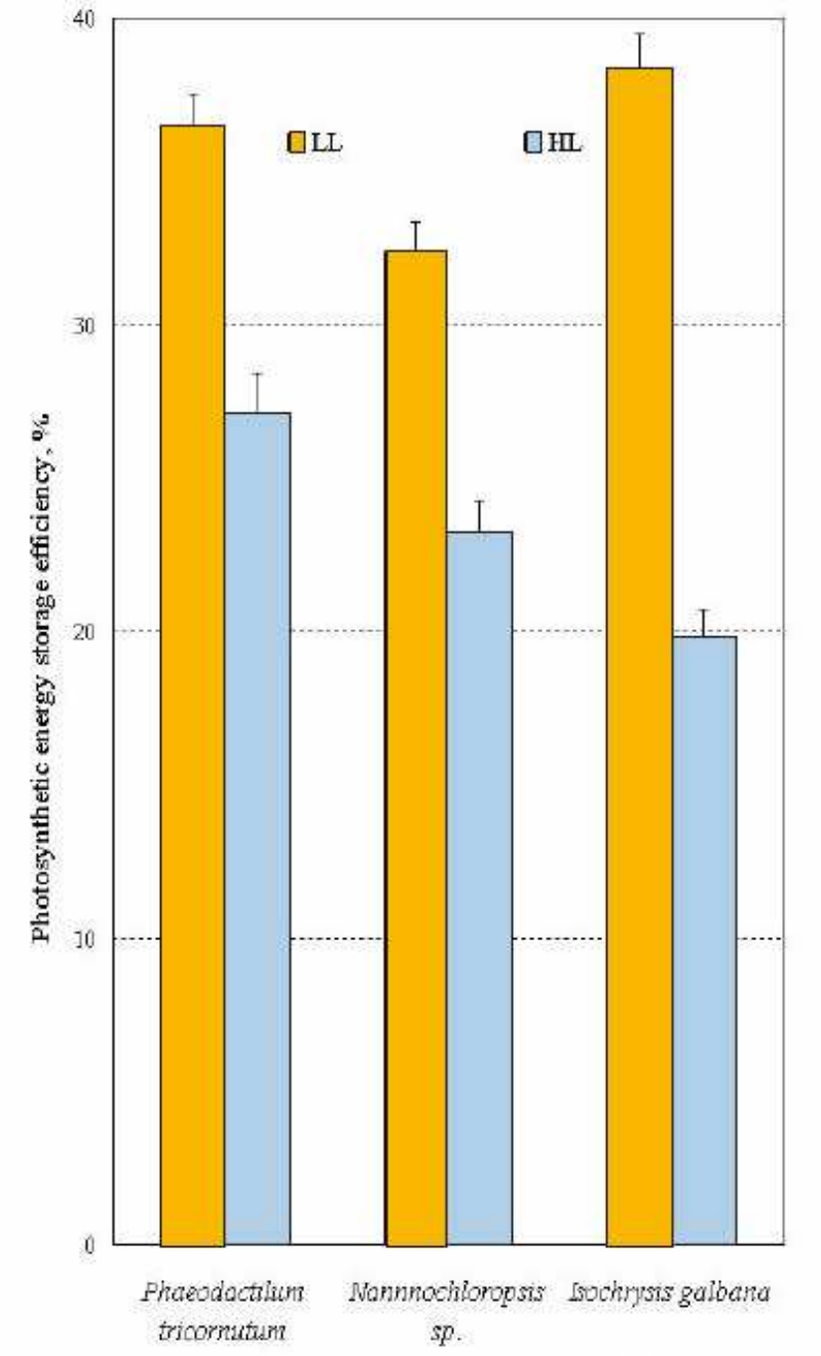

Figure 6. The effect of photoacclimation to high light (500 µmole qm^{-2} s^{-1}) and low light (10 µmole qm^{-2} s^{-1}) on photosynthetic energy storage efficiency for the three algae [according to Pinchasov et al. (2011)].

In general in all samples, photosynthetic efficiency and chlorophyll concentration (photoacoustic signal) decreased with time.

As is evident from Figure 8, macroalgae rapidly exhausted nutrients in the water, and within 190 h, the controls declined to approximately ~50% of the initial values in *U. rigida*. The addition of nutrients slowed down, but did not prevent, such decline (~20 % in *U. rigida*, Fig. 8).

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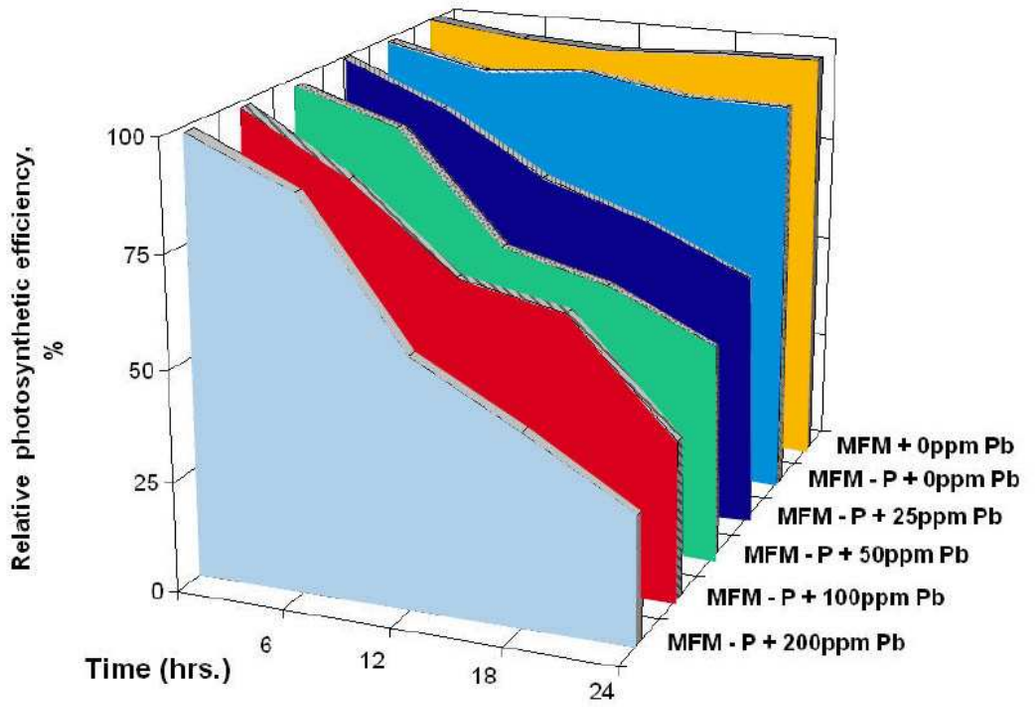

Figure 7. Relative photosynthetic efficiency following application of lead to *Synecococcus leopoliensis* [as per Pinchasov et al. (2006)].

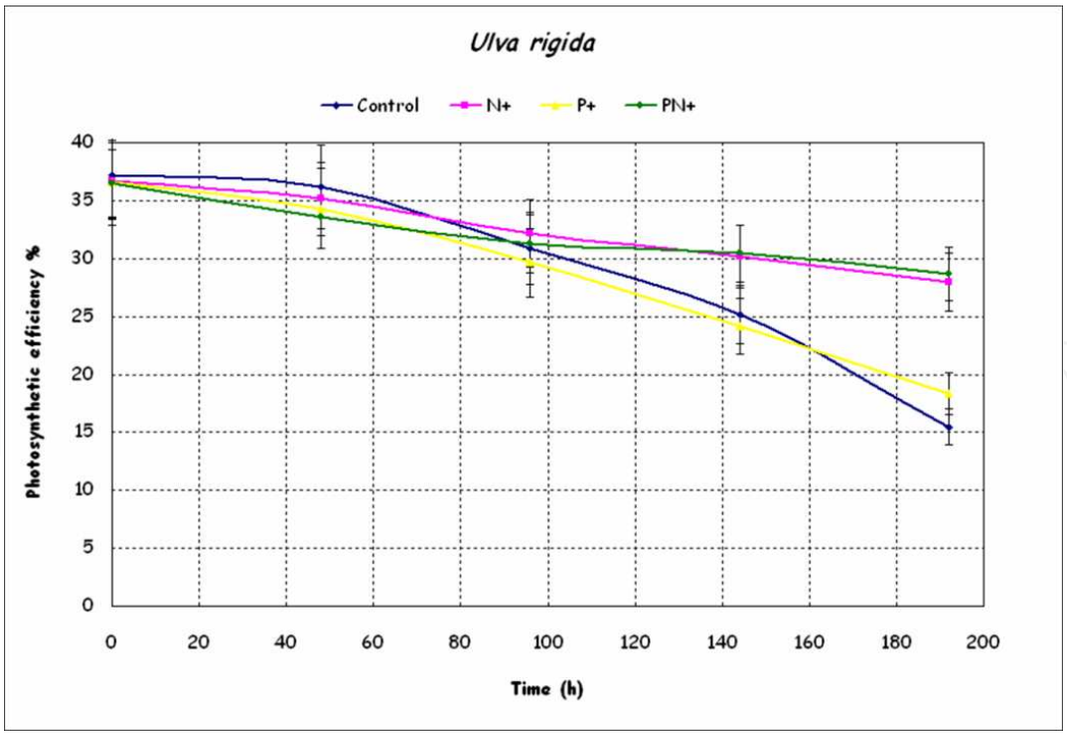

Figure 8. The effect of nutrient enrichment on photosynthetic efficiency of *Ulva rigida*, measured by photoacoustics [according to Pinchasov-Grinblat et al. (2012)].

Recently, Yan et al. (Yan et al., 2011), using a photoacoustic setup, measured thermal dissipation and energy storage in the intact cells of wild type *Chlamydomonas reinhardtii* and mutants lacking either PSI or PSII reaction centers. The photoacoustic signal from PSI-deficient mutants with open reaction centers had a positive phase at 25 °C but a negative phase at 4 °C. In contrast, PSII-deficient mutants showed large negative amplitude at 25 °C and an even larger effect at 4 °C. Kinetic analysis revealed that PSI and PSII reaction centers exhibit strikingly different photoacoustic signals, where PSI is characterized by a strong electrostriction signal and a weak thermal expansion component while PSII is characterized by a small electrostriction component and large thermal expansion (Yan et al., 2011).

9. Other applications of photoacoustics

The thermal expansion of tissue, liquids, and gases due to light energy converted to heat, is termed the photothermal signal. This is always generated when photosynthetic tissue or cell is exposed to a light pulse, since plant tissue never absorbs all of the light stored as products of the process. The unused fraction of the absorbed light energy is converted to heat, resulting in measurable transient pressure (Cahen et al., 1980; Malkin, 1996). In addition to this thermal expansion signal, when a leaf is illuminated by a pulse of light, the resulting photosynthetic photolysis of water causes the evolution of a burst of gaseous oxygen. This process leads to an increase in pressure, a change which is readily detected by a microphone as the photobaric signal. For detailed definitions and description, see review by Malkin (1996).

The photoacoustic technique allows an investigation of energy conversion processes by photocalorimetry and direct measurement of the enthalpy change of photosynthetic reactions (Cahen & Garty, 1979; Malkin & Cahen, 1979). Oxygen evolution by leaf tissue can be measured photoacoustically with a time resolution that is difficult to achieve by other methods (Canaani et al., 1988; Cha & Mauzerall, 1992). A microphone can sense the photoacoustic waves via thermal expansion in the gas phase, thus allowing in-vivo measurements of the photosynthetic thermal efficiency and the optical cross section of the light harvesting systems. O_2 evolution in intact undetached leaves of dark adapted seedlings was measured during photosynthesis with the objective to detect genetic differences among cultivars (da Silva et al., 1995).

The rapid response of the phytoplankton populations to changes in environmental factors, such as temperature, light, nutrients, vertical mixing, and pollution, necessitates simple and frequent measurements. The photoacoustic method provides unique capabilities for ecological monitoring, photosynthesis research, and the optimization of algal mass cultures, such as those designed for the production of biofuel, aquaculture feed, and fine chemicals.

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The authors wish to thank L.P.P. Ltd. Publishers for permission to use Figure 5 (The effect of different iron concentrations on the relative photosynthetic efficiency in the three algae *Nannochloropsis* sp., *Phaeodactylum tricornutum* and *Isocrysis galbana*).

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