

The Effect of Nutrient Enrichment on three Species of Macroalgae as Determined by Photoacoustics

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Abstract The efficiency of phototrophs in storing light energy in products of photosynthesis, or the quantum yield of the process, determines the flux of energy into any ecosystem, and is readily affected by any stress factor, physiological or environmental. Hence it can serve as a sensitive reporter of imminent ecosystem perturbation. Current methods of estimating that parameter are either indirect, or tedious and labor consuming. The photoacoustic method is based on the direct sensing of the variable fraction of light energy degraded to heat, that while absorbed by the plant cells or tissue, failed to be stored as energy rich photosynthate. Algae are the main primary producers in all water bodies, marine, freshwater, natural and manmade, and as such they provide the energy basis for all aquatic ecosystems. The biomass and vitality of the algal population responds rapidly to seasonal changes in environmental factors such as temperature, light, vertical mixing, eutrophication, pollution and nutrient limitation. Typical processes resulting from human activities such as non-point sources of agrochemicals, sewage outflows, and effluents from food, animal and other chemical processing industries as well as from urban and rural runoff, contribute considerable amounts of nitrogen and phosphorus, in many cases in addition to heavy metals, to natural and artificial water bodies. In this study, we examined by the photoacoustic method the effects of enrichment by nitrogen and phosphorus on the photosynthetic efficiency of three common Mediterranean seaweed species which were collected from the field. The application developed by us, allows the rapid and direct determination of phytoplankton photosynthetic activity, by relating the energy stored photochemically to the total light energy absorbed by the plant material. The aim of this work was the examination of the applicability of the photoacoustic system to ecological work with macroalgae, since until now it has been successfully used only in homogenous phytoplankton cultures.

Keywords Photoacoustics, Photosynthesis, Macroalgae, Nutrient Enrichment

1. Introduction

The seasonal changes in algae populations are brought about by the annual rhythms of temperature, light intensity, and enrichment or depletion of nutrients in surface waters [1].

Under sufficient light intensity, photosynthesis of phytoplankton is limited primarily by the availability of essential nutrients, most commonly nitrogen and phosphorus [2-4]. Therefore, any increase or decrease in the supply of these nutrients results in dramatic changes in the concentration of phytoplankton [1],[5]. Such changes in nutrient concentrations, may stem from natural events like mixing of water bodies and upwelling currents, or from anthropogenic sources like marine and lacustrine pollution

[5]. Since even a barely detectable increase in nutrients is amplified by the phytoplankton into an easily quantifiable change in their total biomass and taxonomic composition, phytoplankton are sensitive pollution detectors. The capability to harvest nutrients from the external environment at both high and low concentrations is an important property of the ecology of algae.

These changes in biomass and productivity have been traditionally recorded by measurements of absorption, fluorescence, oxygen evolution, and ¹⁴C labeling [6],[7]. The potential of photoacoustics in quantifying and characterizing algae photosynthesis and chlorophyll concentration had been reported [6],[7]. The photoacoustic method is based on the conversion of the light energy into heat. The application developed by us, allows the rapid and direct determination of algae photosynthetic activity, by relating the energy stored photochemically to the total light energy absorbed by the plant material.

For detailed description see "Methods and Materials". Seaweeds are "benthic algae" that live in the sea. [8].

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Seaweeds are macroalgae which are distributed in three phyla: Chlorophyta which are often called green algae (~1200 species), Rhodophyta, the red algae (~6000 species) and Heterokontophyta or brown algae (~1750 species)[9].

Seaweeds grow in the photic zone which is in the range between the surface water levels (in the intertidal zone) and down to ~200 meters deep in the open ocean[10].

Ulva rigida C. Agardh, *Hypnea musciformis* (Wulfen in Jacquin) J.V. Lamouroux and *Padina pavonica* (Linnaeus) Thivy Agardh are green, red and brown algae respectively. These three macroalgae are very common along the Israeli shore and mainly grow on the Kurkar intertidal abrasion platforms.

The rationale for the species selection was to compare the effects of the unique laser used previously[11],[12] to estimate photosynthesis efficiency only on phytoplankton, on species of macroalgae from three different phyla that have different types of thallus morphologies and diverse pigment assortments. *Ulva rigida* has green flat sheet-like thallus with toothed margins. This thallus is composed of two cell layers[13]. *Hypnea musciformis* has red-green narrow, cylindrical, branched thallus morphology. Its branch apices are slightly upcurved, flattened hooks[14]. *Padina pavonica* is characterized by flat calcified "ear-like" blades which has circinnately inrolled apical margins[15].

The aim of this work was the examination of the applicability of the photoacoustic system, previously used only in homogenous phytoplankton cultures, to ecological work with macroalgae.

2. Methods and Materials

2.1. Photoacoustics

The photoacoustic method is based on the conversion of absorbed light to heat. When the energy of the photons absorbed in a sample is degraded to heat the thermal expansion of the material causes a volume increase:

$$\Delta V = \alpha \Delta H \div C_p \rho \quad (1)$$

where α is the thermal expansivity, $\alpha = 1 \div V (\partial V \div \partial T)_p$, ρ , the density, C_p , the heat capacity of the medium and ΔH is the heat liberated or enthalpy change[16],[17].

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[6],[11],[18].

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 larger will be the difference between the stored energy with and without ongoing photosynthesis[19].

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 photosynthetic storage efficiency, Φ 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 illumination (PA_{light})[7],[11].

$$\Phi = (PA_{\text{light}} - PA_{\text{dark}}) \div PA_{\text{light}} = 1 - PA_{\text{dark}} \div PA_{\text{light}} \quad (2)$$

We detect and follow the pressure wave in an aqueous suspension of algae over 10-20 microseconds after the laser pulse.

The experimental setup:

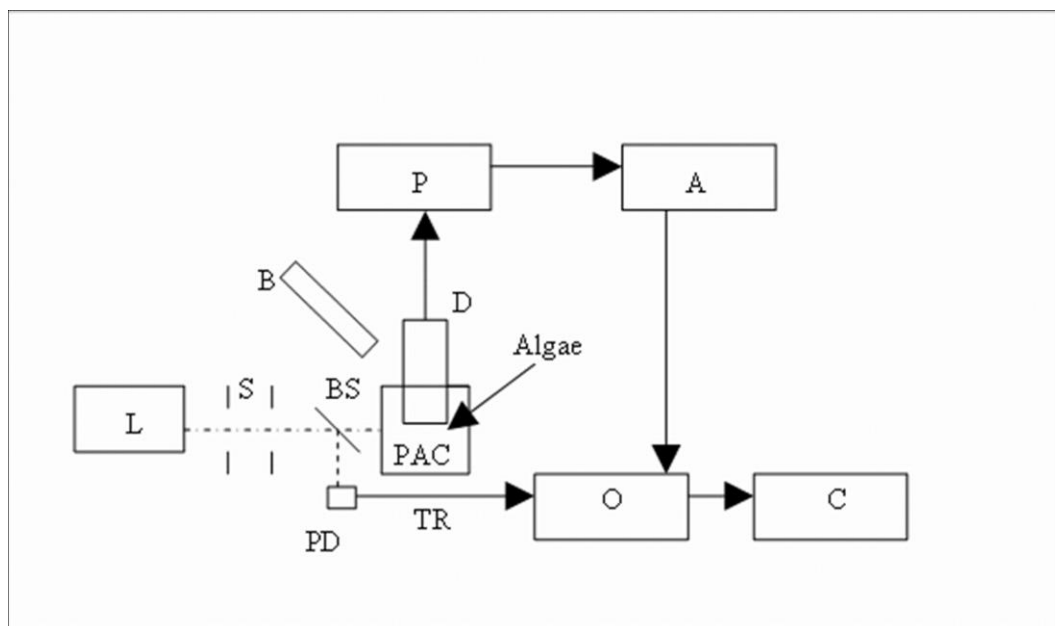


Figure 1. The schema of the photoacoustic system. L - Minilite Q - Switched Nd :YAG Laser, 532 nm; S - beam-shaping slits; BS - beam splitter; PAC - photoacoustic cell with suspension of algae (30 ml); D - stainless-steel photoacoustic detector, contained a 10-mm-diameter resonating ceramic disc (BM 500, Sensor, Ontario, Canada); P - low-noise Amptek A-250 preamplifier; A - SRS 560 - low noise amplifier; PD - photodiode; TR - trigger signal; B - background light source, quartz-halogen illuminator (Cole Parmer 4971); O - Tektronix TDS 430A oscilloscope; and C - computer[11],[12]

The experimental setup is shown schematically in Figure 1. The overall procedure was similar to that described in detail in Pinchasov et al.[11]. The second harmonic of a Continuum Minilite Q-Switched Nd-Yag laser at 532 nm was used. The signal was processed with a Tektronix TDS 430A oscilloscope. The submersible, stainless steel enclosed homemade detector contained a 10 mm diameter resonating ceramic disc (BM 500, Sensor, Ontario, Canada). The sample was placed in a 16 mm quartz glass cell (PAC). The laser (L) pulse, after passing through a pair of 1 mm wide slits (S) is incident upon the suspension of algae whose pigments absorb part of the laser light. 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 producing an acoustic wave. This is intercepted by a detector (D), containing the above ceramic disc. A small portion of the laser pulse is deflected by a beam splitter (BS) and used to trigger the Tektronix TDS 430A oscilloscope, where the amplified (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. Signal to noise was improved by averaging over 128 pulses, and by taking RMS values over the time of the recorded signal ($\sim 10 \mu\text{s}$). Weak ($\sim 20 \mu\text{J cm}^{-2}$), 5 ns pulses at 532 nm wavelength, were used as a probe for ongoing photosynthesis. The source of the background light (B), was a quartz-halogen illuminator (Cole-Parmer 4971). The intensity of the background light was adjusted to the $2000 \mu\text{E m}^{-2} \text{s}^{-1}$ by neutral density filters and measured with a LiCor light meter equipped with a cosine quantum sensor (for technical details see Pinchasov et al.[11]).

2.2. Algae Samples

The samples of three common macroalgae species: *Ulva rigida*, *Hypnea musciformis* and *Padina pavonica* were collected from the intertidal abrasion platforms at Bat Yam (located in the middle of the Israeli Mediterranean) during spring 2010.

All samples were kept at $22 \pm 0.1^\circ\text{C}$ in 100 mL Erlynmayers during 192 hours under continuous irradiance at $\sim 200 \pm 5.0 \mu\text{J m}^{-2} \text{s}^{-1}$. Initial volume of medium in Erlynmayers was 75 mL. For every species were processed 3 duplicate samples.

Chlorophyll was determined by photoacoustic method, based on the proportionality of the photoacoustic signal to the amount of pigment. For all experiments (include control) were used slices of common.

The samples were exposed to 3 treatments: nitrogen (was added as NaNO_3 , 10 mL at concentration of 50 mL/L from the stock of 75.0 g/L), phosphorus (was added as NaH_2PO_4 , 10 mL at concentration of 5 mL from the stock of 5.0 g/L), and nitrogen and phosphorus together. Controls were kept in seawater alone.

3. Results and Discussion

We have used the effects of nutrient limitation and of eutrophication on the photosynthetic efficiency of seaweeds as experimental variables in a demonstration of the applicability of photoacoustics in aquatic ecology. 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.

Since nutrient status is known to rapidly affect algal growth and photosynthesis we chose that environmental factor for our study

In general, in all samples (Figs. 2, 3 and 4), photosynthetic efficiency and chlorophyll concentration (photoacoustic signal) decreased with time, during the 192 hours of incubation..

As is evident from Figures 2, 3 and 4, the photoacoustic signal rapidly decreased, and within 190 hours, the controls declined to some 70 % in *P. pavonica* (Fig. 2) and *H. musciformis* (Fig. 4), and ~ 50 % in *U. rigida* (Fig. 3), of the initial values. The addition of nutrients slowed down, but did not prevent, such decline (~ 20 % in *U. rigida* (Fig. 3) and ~ 50 % and 30 % in *P. pavonica* and *H. musciformis*, respectively (Figs. 2 and 4). The decline in all treatments, including in the P and N enriched ones is likely due to the requirement for additional nutrients besides nitrogen and phosphorus.

Similar effects of nutrient limitation on photosynthetic apparatus and chlorophyll concentration have been reported by Villares et al.[20] and Litchman et al.[21]. Our results suggest that the added nitrogen prevented this nutrient from becoming limiting, and, therefore, the observed declines in photosynthesis and chlorophyll were due to the incipient phosphorus limitation.

Samples with added nitrogen and phosphorus together were usually the slowest to show a decrease in photosynthetic efficiency.

A decrease in chlorophyll concentration under nutrient limitation has been observed for both marine and freshwater algae[21-23].

Reduction trend in chlorophyll concentration in nutrient-enriched samples may be explained by the availability of nutrients required for the synthesis of pigments (mostly nitrogen), and membranes (both nitrogen and phosphorus).

The general conclusion from these data, which was obtained by photoacoustics, is that in the sampled algae, nitrogen was the first nutrient that became limiting followed by phosphorus. In all cases the addition of phosphorus and nitrogen alone, or together slowed down the decline in photosynthetic efficiency, that was the fastest in the unsupplemented controls.

Our results, which were obtained by the photoacoustic method, agree with others, previously described in literature [24-26] showing that the nitrogen and the phosphorus additions mitigated the effects of nutrient limitation.

Our results show that photoacoustics can be a useful tool in ecophysiological studies of macroalgae, providing sensitive and immediate evidence for any deleterious

processes affecting seaweeds. Based on our results we are working on expanding the method's applicability to various additional phototrophs such as seagrasses, turf algae, algal mats and zooxanthellate corals..

The directness of our method holds the potential for development of novel tools for monitoring changes in

photosynthetic efficiency related to water quality in marine and freshwater benthos communities. The photoacoustic method can provide early warning alerts to incipient negative ecosystem developments, as well as report recovery processes in response to the implementation of remediation measures

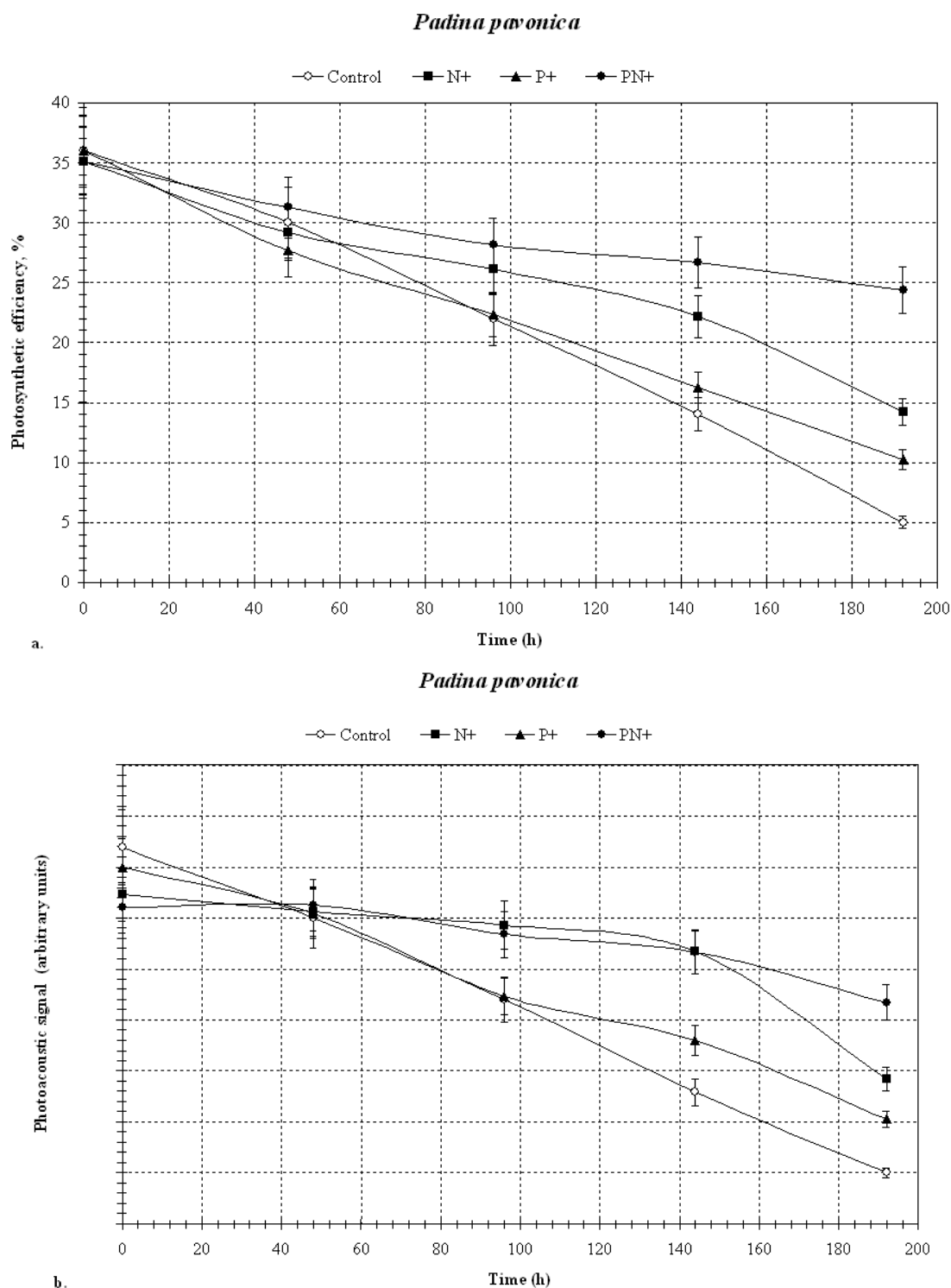


Figure 2. *P. pavonica* – photosynthesis (a) and chlorophyll (b) by photoacoustics

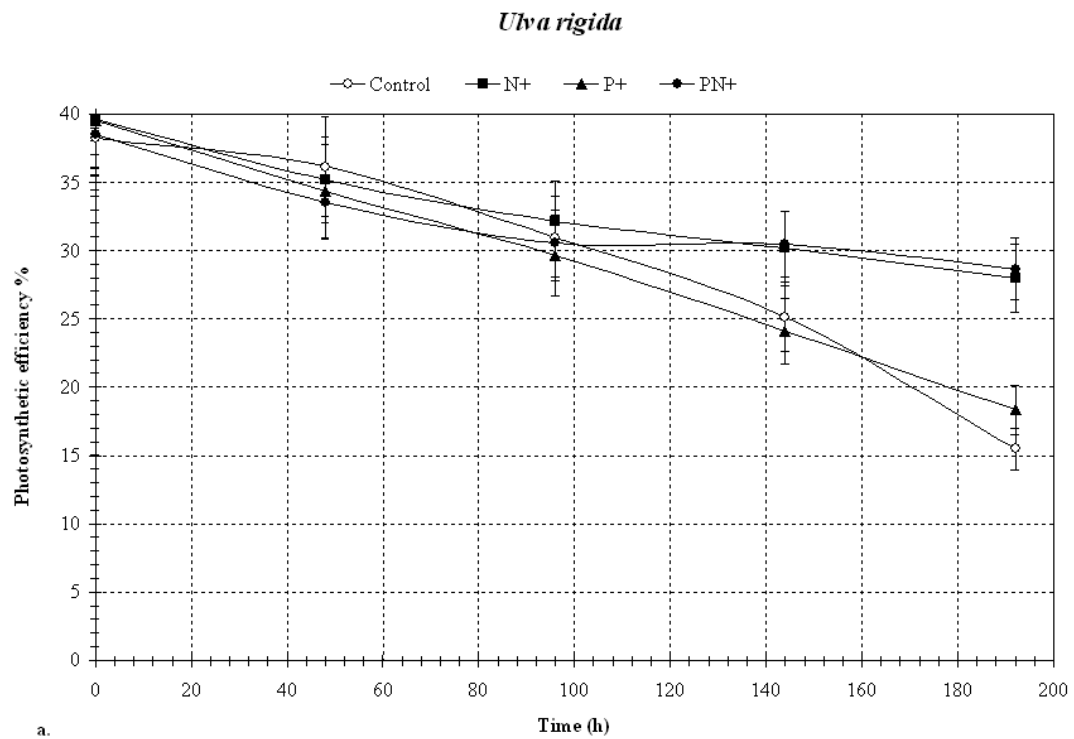
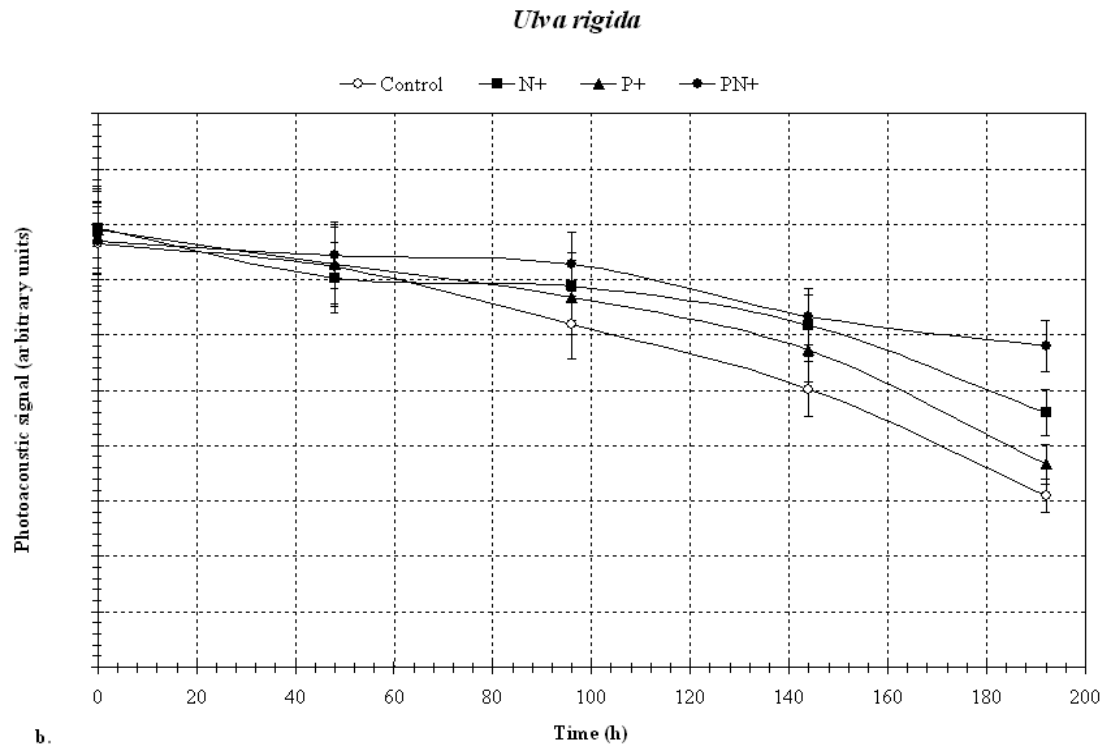


Figure 3. *U. Rigida* – photosynthesis (a) and chlorophyll (b) by photoacoustics

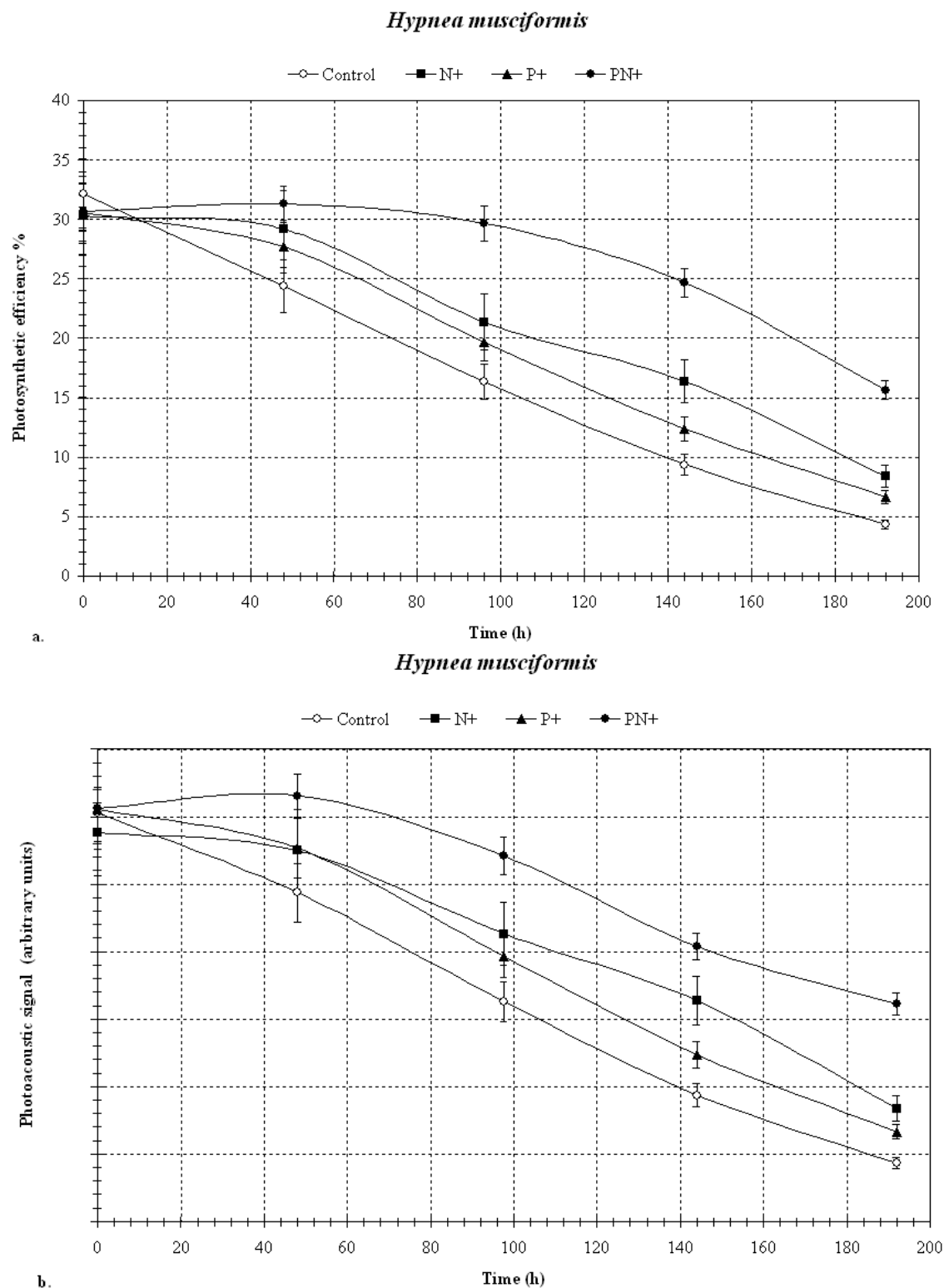


Figure 4. *H. musciformis* – photosynthesis (a) and chlorophyll (b) by photoacoustics

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REFERENCES

- [1] Dubinsky Z, Productivity of algae under natural conditions, ed. Richmond A. Handbook for Algal Mass Culture. CRC, pp. 107-116, 1986.
- [2] Berger WH, Smetacek WS, Wefer G, Productivity of the Ocean: Present and Past. Wiley & Sons, New York, 1989.

- [3] Cullen J, Yang X, MacIntyre HL, Nutrient limitation and marine photosynthesis, eds. Falkowski PG, Woodhead AD. Primary Productivity and Biogeochemical Cycles in the Sea. Plenum Press, New York, pp. 69-88, 1992.
- [4] Dugdale R, Wilkerson F, Nutrient limitation of new production in the sea, eds. Falkowski PG, Woodhead AD. Primary Productivity and Biogeochemical Cycles in the Sea. Plenum Press, New York, pp. 107-122, 1992.
- [5] Dubinsky Z, Stambler N, "Eutrophication, marine pollution and coral reefs", *Global Change Biology*, vol. 2, pp. 511-526, 1996.
- [6] Dubinsky Z, Feitelson J, Mauzerall DC, "Listening to phytoplankton: Measuring biomass and photosynthesis by photoacoustics", *Journal of Phycology*, vol. 34, pp. 888-892, 1998.
- [7] Mauzerall DC, Feitelson J, Dubinsky Z: "Discriminating between phytoplankton taxa by photoacoustics", *Israel Journal of Chemistry*, vol. 38, pp. 257-260, 1998.
- [8] Guiry MD, <http://www.seaweed.ie/algae/seaweeds.html> (viewed 6 November 2011).
- [9] Guiry MD: <http://www.seaweed.ie/> (viewed 6 November 2011).
- [10] Graham LE, Wilcox LW, *Algae*. Benjamin, Redwood City, 2000.
- [11] Pinchasov Y, Kotliarevsky D, Dubinsky Z, Mauzerall DC, Feitelson J, "Photoacoustics as a diagnostic tool for probing the physiological status of phytoplankton", *Israel Journal of Plant Sciences*, vol. 53, pp. 1-10, 2005.
- [12] Pinchasov Y, Porat R, Zur B, L. S, Dubinsky Z, "Photosynthetic efficiency as function of nutrient status in phytoplankton from several irrigation and drinking water reservoirs, determined by photoacoustics", *Israel Journal of Plant Sciences*, vol. 56, pp. 69-74, 2008.
- [13] Brodie J, Maggs CA, John DM, eds., *Green Seaweeds of Britain and Ireland*, British Phycological Society, pp. 2007.
- [14] Won BY, Yates KK, Fredericq S, Cho TO, "Characterization of macroalgal epiphytes on *Thalassia testudinum* and *Syringodium filiforme* seagrass in Tampa Bay, Florida", *Algae*, vol. 25, pp. 141-153, 2010.
- [15] Geraldino PJL, Liao LM, Boo SM, "Morphological study of the marine algal genus *Padina* (Dictyotales, Phaeophyceae) from Southern Philippines: 3 species new to the Philippines", *Algae*, vol. 20, pp. 99-112, 2005.
- [16] Feitelson J, Mauzerall DC, "Wide band time-resolved photoacoustic study of electron transfer reactions: Difference between measured enthalpy and redox free energies", *Journal of Physical Chemistry*, vol. 97, pp. 8410-8413, 1993.
- [17] Mauzerall D, Feitelson J, Prince R, "Wide band, time-resolved photoacoustic study of electron transfer reactions: difference between measured enthalpies and redox free energies", *Journal of Physical Chemistry*, vol. 99, pp. 1090-1093, 1995.
- [18] Malkin S, The photoacoustic method in photosynthesis-monitoring and analysis of phenomena which lead to pressure changes following light excitation, eds. Ames J, Hoff AD. *Biophysical Techniques in Photosynthesis*. Kluwer Academic Publishers, Dordrecht, pp. 191-206, 1996.
- [19] Cha Y, Mauzerall DC, "Energy storage in linear and cyclic electron flows in photosynthesis", *Plant Physiology*, vol. 100, pp. 1869-1877, 1992.
- [20] Villares R, Puente X, Carballeira A, "Nitrogen and phosphorus in *Ulva* sp. in the Galician Rias Bajas (northwest Spain): Seasonal fluctuations and influence on growth", *Boletín Instituto Español de Oceanografía*, vol. 15, pp. 337-341, 1999.
- [21] Litchman E, Steiner D, Bossard P, "Photosynthetic and growth responses of three freshwater algae to phosphorus limitation and daylength", *Freshwater Biology*, vol. 48, pp. 2141-2148, 2003.
- [22] Geider RJ, MacIntyre HL, Graziano LM, McKay RML, "Responses of the photosynthetic apparatus of *Dunaliella tertiolecta* (Chlorophyceae) to nitrogen and phosphorus limitation", *European Journal of Phycology*, vol. 33, pp. 315-332, 1998.
- [23] Wykoff DD, Davies JP, Melis A, Grossman AR, "The regulation of photosynthetic electron transport during nutrient deprivation in *Chlamydomonas reinhardtii*", *Plant Physiology*, vol. 117, pp. 129-139, 1998.
- [24] Wehr JD, "Experimental tests of nutrient limitation in freshwater picoplankton", *Applied and Environmental Microbiology*, vol. 55, pp. 1605-1611, 1989.
- [25] Pedersen MF, Borum J, "Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake", *Marine Ecology-Progress Series*, vol. 161, pp. 155-163, 1997.
- [26] Larned ST, "Nitrogen- versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae", *Marine Biology*, vol. 132, pp. 409-421, 1998.