

Photoacoustics: a novel application to the determination of photosynthetic efficiency in zooxanthellate hermatypes

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Abstract

We present here a novel application of photoacoustics to the monitoring and study of the physiological status of zooxanthellate corals. Until now, the method has only been applied in homogeneous phytoplankton cultures and field samples. Corals are among the world's most productive ecosystems because they host symbiotic dinoflagellates (zooxanthellae) that provide them with a large amount of photosynthates for their energy requirements. Coral reefs face unprecedented pressures on local, regional, and global scales due to climate change and anthropogenic disturbances. Responses to such stress are often a decrease in the photosynthetic efficiency of the symbiotic dinoflagellates, as well as bleaching, which involves the mass expulsion of these symbionts or loss of their pigments. Photosynthesis is a sensitive indicator of stress in plants and plays a central role in the nutrition of symbiotic invertebrates. Our aim was to examine the applicability of photoacoustics, developed by us for ecological work with phytoplankton, to the study of symbiotic dinoflagellates in situ. We have determined areal chlorophyll content and light energy storage efficiency with three zooxanthellate coelenterates, two corals and one hydrozoan, hosting symbiotic algae. We also present the effect of temperature elevation on the decrease in photosynthetic efficiency of the symbiotic coral *Stylophora pistillata* determined by photoacoustics. Our results demonstrate the potential, power, and convenience of photoacoustics in following bleaching-related changes in coral pigmentation, in the photosynthetic energy storage efficiency of corals, and in its usefulness in diagnosing its health in relation to environmental factors, in the example presented here, seawater warming.

The anthropogenic increase in atmospheric CO₂ causes two global climate change related processes in the marine domain, seawater warming and ocean acidification, both well documented (Fine and Tchernov 2007; Dubinsky and Stambler 2009; Rodolfo-Metalpa et al. 2011). Both of these have significant adverse effects on reef building corals. Sea warming affects corals by increasing both the frequency and severity of coral bleaching events (Hoegh-Guldberg 2011; Doropoulos et

al. 2012), whereas the decrease in seawater pH interferes with the skeletal calcification (Fine and Tchernov 2007). The well-being of reef building corals depends on their mutualistic symbiosis with the endocellular symbionts, the zooxanthellae (Stambler 2011), which supply most of the energy required by host and symbiont. Hence, it is of great interest and importance to be able to detect any incipient decline in the efficiency of the photosynthetic light use efficiency of the zooxanthellae. As we have previously demonstrated in work with phytoplankton, photoacoustics provides a direct and fast way to determine photosynthetic efficiency (Dubinsky et al. 1998; Mauzerall et al. 1998; Pinchasov et al. 2006, 2007; Pinchasov-Grinblat et al. 2010). However since previous work was done on homogeneous algal cultures or assemblages, the complex geometry of coral colonies, their rough and uneven surface required examination and adaptation of methodology, which resulted in excellent results, presented here.

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Photosynthetic efficiency: Definitions

The determination of photosynthetic efficiency is complicated by both theoretical and experimental difficulties. Any definition of efficiency consists of a ratio, or fraction of the desired product divided by the invested energy, both of these measured for the same time interval (Eq. 1).

$$\text{photosynthetic efficiency} = \frac{\text{biomass increment, evolved oxygen, assimilated CO}_2}{\text{absorbed light energy}} \quad (1)$$

In photosynthesis, both the numerator and the denominator of that fraction are ambiguous. As far as the numerator, which ideally should include all stored light energy [photosynthetically stored radiation, PSR (Morel et al. 1987)], is concerned, the following were considered and used: biomass increment over some area and time, oxygen evolved, and carbon assimilated. The biomass increment fails in some cases to account for losses due to grazing and parasites, in all cases it represents net photosynthesis (P_N)—requiring correction for respiration (Eq 2), losses of excreted or exudated dissolved organic carbon compounds, and energy stored by PSI activity as ATP generated by both cyclic and non-cyclic photophosphorylation.

$$P_N = \text{Gross photosynthesis} - \text{Respiration} = P_G - R \quad (2)$$

Whereas oxygen evolution does include the equivalent of the excreted DOC (dissolved organic carbon), it shares all other shortcomings, as does also the assimilated carbon. The units used, moles or their energy equivalent, will also affect the efficiency values. The denominator also poses several difficulties: whereas photobiologists and photochemists strive to use only the light energy absorbed by the plant pigments [PUR, (Morel et al. 1987)], ecologists are interested in the photosynthetically available radiation [PAR, (Morel et al. 1987)], or the flux of light impinging on a given area per unit time (Eq. 3, for a review and discussion of terms and definitions, see Dubinsky and Berman (1976) and Dubinsky (1980)).

In all cases, the calculated efficiency depends on whether the light energy is expressed as quanta or as its wavelength-dependent energy equivalent (Eq. 3).

$$E = h\nu \quad (3)$$

Whenever the product of gross photosynthesis is determined as moles of oxygen evolved or of carbon assimilated, and light absorbed is measured as moles of photons, their ratio becomes the quantum yield ϕ (Eq.4).

$$\phi = \frac{\text{moles O}_2 \text{ evolved OR moles CO}_2 \text{ assimilated}}{\text{moles of photons absorbed}} \quad (4)$$

All quantities (Eqs 1, 3, and 4) have to be determined for the same sample.

In phytoplankton cultures and natural communities, the numerator of the quantum yield fraction has been estimated either from ^{14}C assimilation data (e.g., Dubinsky and Berman 1976; Iluz et al. 2008) or oxygen evolution rates (Falkowski et al. 1985). The denominator has been estimated from the absorption of light in successive water layers in a lake or that absorbed in the passage through the measuring cuvette.

Since the development of “pump and probe” variable fluorescence-based methods (Mauzerall and Malley 1971; Cosgrove and Borowitzka 2011), quantum yields of photosynthesis were calculated and estimated based on the kinetics of the rise and decline of fluorescence. Due to the availability of commercial instruments (Fast Rate Repeating Fluorometer, Chelsea Instruments, Fire, Satlantic, PAM, Walz), these methods have become popular. However, they have their problems: fluorescence is due to a small fraction of the absorbed light energy, is an inverse proxy of photosynthesis, room-temperature fluorescence is only emitted by PSII, and the signal is affected by additional factors complicating their translation into standard quantum yield values (Cosgrove and Borowitzka 2011).

Attempts to estimate quantum yields of zooxanthellae corals are even more limited. The photosynthetic production of the algal symbionts in corals, the zooxanthellae, was estimated from oxygen evolution, whereas the absorbed light was determined from the difference between incident and reflected light from the coral (Wyman et al. 1987; Dubinsky et al. 1990), and from the absorbed light when the entire coral colony was enclosed in an integrating sphere (Stambler and Dubinsky 2005). In these three studies, the difference in photosynthetic efficiency for oxygen formation between coral colonies acclimated to high light and extremely low light was found to range between the maximum possible $\phi = 1/8$ in low light acclimated corals to $\phi = 1/100$ in shallow water, high light colonies.

Photoacoustics

The 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:

$$\Delta V = \alpha \Delta H / C_p \rho + \Delta V_{pc} \quad (5)$$

where α is the thermal expansivity, $\alpha = 1/V (\delta V / \delta T)_p$, ρ , the density, C_p , the heat capacity of the medium, and ΔH is the heat liberated or enthalpy change (Feitelson and Mauzerall 1993; Mauzerall et al. 1995a, 1995b). The ΔV_{pc} term is the inherent volume change of the photoinduced photosynthetic reactions over the time range observed. Its major component at short times is electrostriction upon charge formation in the primary electron transfer reactions.

The time derivative of the volume change, via the compressibility κ of the material

($\kappa = -1/V(\delta V/\delta P)_P$), causes a pressure wave that is detected by a suitable transducer. Since we are presently not interested in the time dependence of the process, the detectors used here were piezoelectric ceramics, which are resonant acoustic cavities.

They have a high sensitivity at their resonant frequency but acting as a narrow band filter, they are useful over a restricted range of frequencies. Thin piezoelectric films that we had used previously (Dubinsky et al. 1998; Mauzerall et al. 1998) are broad band detectors and hence span a wide time domain, but they are less sensitive than the ceramics.

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 (Malkin et al. 1990; see review Malkin 1996). Its overwhelming advantage is that it responds only to absorbed light. Scattered light, the bane of all absorption methods, is irrelevant. 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 (Cha and Mauzerall 1992). 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, PS_{max} , is determined as the complement of the ratios of the PA signal, generated by a weak pulse of light in the dark (PA_{dark}), to that obtained under strong continuous illumination (PA_{sat})

$$PS_{(max)} = (PA_{sat} - PA_{dark})/PA_{sat} = 1 - PA_{dark}/PA_{sat} \quad (6)$$

For development of equations and correction for fluorescence, see Cha and Mauzerall (1992). Hitherto, the method has been successfully applied in water to homogenous phytoplankton suspensions such as algal cultures and samples from natural water bodies. In these studies, the pressure wave was detected over 10-20 the microseconds, a time domain over which the signal-to-noise ratio is optimal for the resonant detector.

The complex morphology of corals and their rugose surface posed problems regarding the quality of the expansion wave generated by the laser pulse. We describe here how these difficulties were resolved, resulting in a convenient, reproducible signal, faithfully reporting the status of the algal symbionts embedded in the coral's tissue.

Materials and procedures

Experimental setup

The overall procedure was similar to that described in detail in Dubinsky et al. (1998) and in Mauzerall et al. (1998). 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)

The experimental setup is shown schematically in Fig. 1. For samples, we used 2-5 cm long branches "nubbins" of three zooxanthellae containing reef builders, the corals *Stylophora pistillata* and *Acropora* sp., and the hydrocoral *Millepora dichotoma*. Each sample was placed in a seawater filled 16 mm square glass cell (PA), perpendicular to the laser beam. The laser (L) beam, (S) is incident upon the sample where the pigments of the symbiotic algae, the zooxanthellae, absorb part of the laser light. The beam was adjusted to cover a circular area of ~ 5 mm diameter. Depending on the experimental conditions (see below), 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 the hydrophone (D), containing the above ceramic disc and amplified (Amptek A-250 Preamp and Stanford Research 560 amplifier). The photoacoustic signal is recorded on the Tektronix TDS 430A oscilloscope that is triggered by the laser pulse generator. A series of 32-128 pulses in which the sample was in the dark, were alternated by an identical series of signals, when the sample branch is concomitantly illuminated by saturating continuous light. The source of the background light E, was a quartz-halogen illuminator (Cole-Parmer 4971). We used the RMS values over the time of the recorded signal (~ 10 μ s), after each laser pulse.

Efficiency of photosynthesis and photosynthesis versus energy relationship

To obtain a photosynthesis versus energy relationship, the intensity of the background light was adjusted to the desired level by neutral density filters and measured with a LiCor light meter equipped with a cosine quantum sensor (Pinchasov et al. 2006, 2007; Pinchasov-Grinblat et al. 2010; Pinchasov-Grinblat and Dubinsky 2010).

We were able to determine the efficiency of photosynthesis under different ambient irradiance levels, resulting in a complement energy storage curve (Fig. 2). This relationship is similar in shape to the photosynthesis versus energy (or irradiance), P versus E (or I) curve obtained by the tedious standard measurements of 14 C fixation or oxygen evolution (Berner et al. 1986; Grobbelaar et al. 1992), or the indirect results from measurement of variable fluorescence (Falkowski and Laroche 1991; Schreiber and Bilger 1993).

By increasing the continuous background light intensity (E) 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 (PA_E). From these detector responses, the photosynthetic energy-storage versus background light-intensity relationship was obtained. PS_E is the fraction of maximal photosynthetic

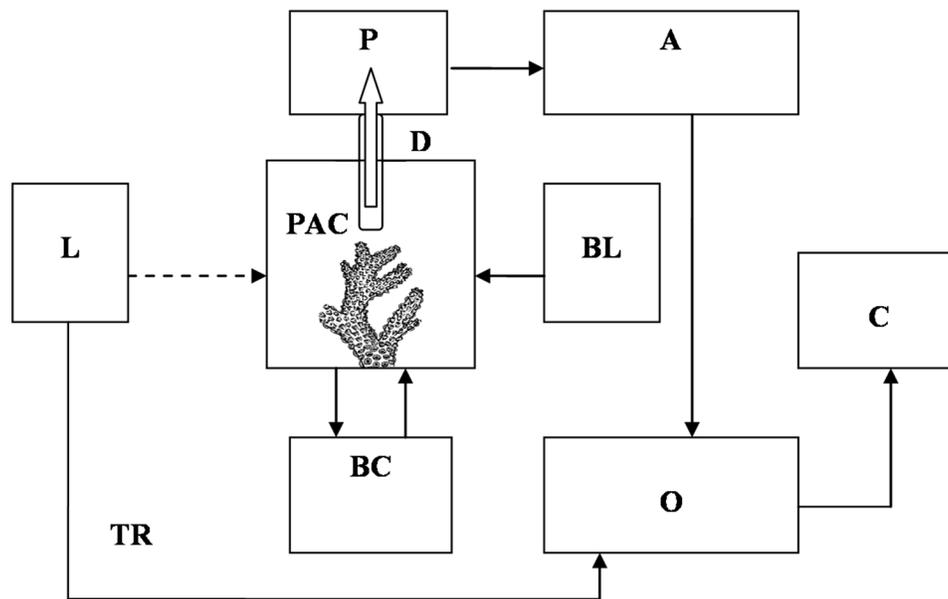


Fig. 1. The scheme of the photoacoustic system. L: Minilite Q – Switched Nd :YAG Laser, 532 nm; PAC: photoacoustic cell with coral fragment; D: stainless-steel photoacoustic detector, contained a 10-mm-diameter resonating ceramic disc (BM 500, Sensor, Ontario, Canada); P: low-noise Amptek A-250 preamplifier; A: SRS 560 – low noise amplifier; TR: trigger signal; BL: background light source, quartz-halogen illuminator (Cole Parmer 4971); BC: heating bath circulator CW-G; O: Tektronix TDS 430A oscilloscope; and C: computer.

energy storage efficiency at a given continuous background light intensity E , as described in our previous, Mauzerall et al. (1998) communication,

$$PS_E = (PA_E - PA_{\text{dark}}) / (PA_{\text{sat}} - PA_{\text{dark}}) \quad (7)$$

where “sat” and “dark” mean with and without saturating background illumination. To normalize the results, we converted the absolute energy storage efficiency to maximal one.

Coral samples

The coral samples, collected according to Israeli Parks Authority permits, from the Gulf of Eilat (Aqaba) were kept in the lab in running sea water at 24°C, under white fluorescent lights at $\sim 220 \mu\text{mole quanta m}^{-2} \text{ s}^{-1}$ of PAR.

Areal chlorophyll concentration

The areal chlorophyll, for the comparison of the values based on photoacoustics to standard procedures was obtained as follows. After the photoacoustic measurements, the tissue from the entire nubbin was stripped by air brush and algal cells in the resulting slurry were counted under the microscope in a Neubauer cytometer. The area of the nubbin was determined by the aluminum foil method. Chlorophyll *a* was determined spectrophotometrically following overnight extraction with dimethyl formamide of centrifuged cells at room temperature, according to the equations (Jeffrey and Humphrey 1975). From these values the areal chlorophyll concentrations ($\mu\text{g chlorophyll cm}^{-2}$) and zooxanthellae densities (cells cm^{-2}) were calculated.

The laser beam was aimed at the nubbin as to cover a cir-

cular area of $\sim 5 \text{ mm}$ diameter, thereby averaging the small scale differences in zooxanthellae distributions in the different polyp parts and the coenosarc areas between these small polyp species. The results were calibrated against extracted chlorophyll (Fig. 3).

Photosynthetic light use efficiency determinations

The values derived by photoacoustics from healthy organisms (Fig. 4) are likely to present maximal values and are higher than most reported for various aquatic phototrophs approaching only values obtained under dim light (Dubinsky 1980; Dubinsky et al. 1990; Schanz et al. 1997).

Assessment and discussion

In general, there are several reasons why relatively high efficiencies are to be expected from the photoacoustic measurements. The photoacoustic signal is detected prior to the electron migration through the quinone pool requiring some 1.5-15 ms, and additional energy dissipating steps taking place before the evolution of oxygen and the assimilation of CO_2 . Furthermore, as has been pointed out (Herzig and Dubinsky 1993), PSI activity alone, such as cyclic photophosphorylation does not produce tangible entities that can be quantified from oxygen evolution or carbon assimilation but do so in PA, where they are included. Also, photoacoustic data are gathered from gross photosynthesis, as respiratory consumption of photosynthate takes place later on.

The comparison between chlorophyll estimated by photoacoustics in vivo, and extracted values determined photospectrometrically show good correlation ($R^2 = 0.9775$), Fig. 3 show-

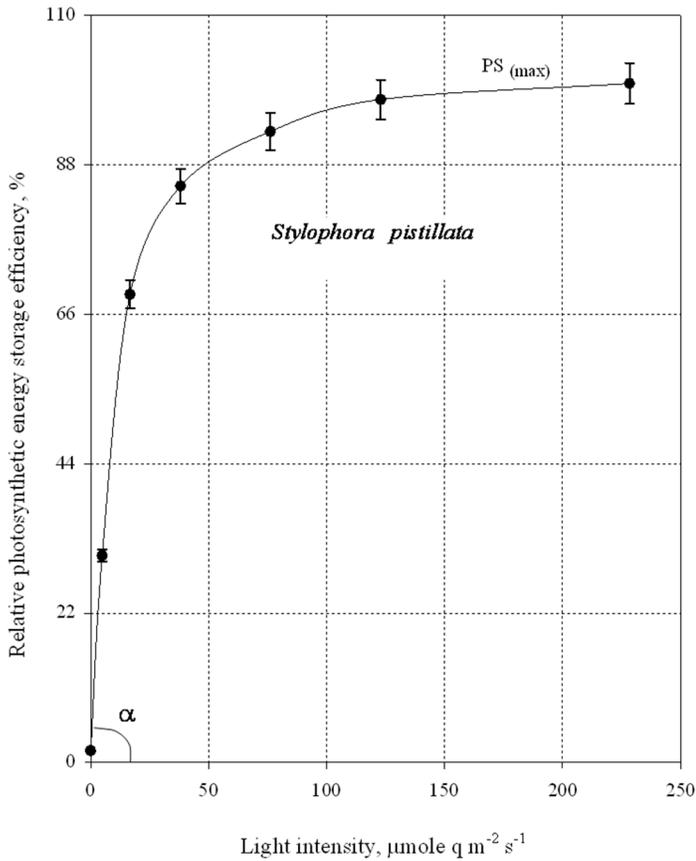


Fig. 2. Complement of the relative photosynthetic energy storage efficiency (versus light irradiance for symbiotic coral *Stylophora pistillata*). Photoacoustic signal from 3 different corals' fragments (*Stylophora pistillata*, *Millepora dichotoma*, *Acropora* sp.), whereas PAS in "dark" is the photoacoustic signal generated by a laser pulse in the dark and PAS in "light" is a signal obtained under saturating continuous light. The area between the two lines is proportional to the fraction of the pulse's light energy stored as products of photosynthesis.

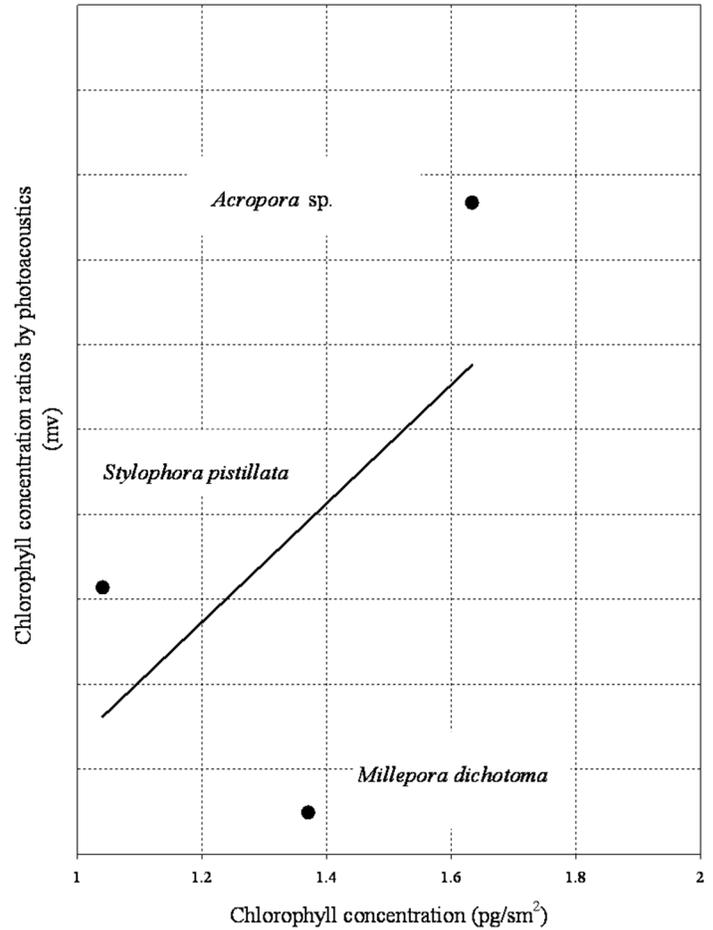


Fig. 3. Chlorophyll concentration by photoacoustics versus chlorophyll by standard method for symbiotic corals *Acropora* sp., *Stylophora pistillata*, and *Millepora dichotoma*.

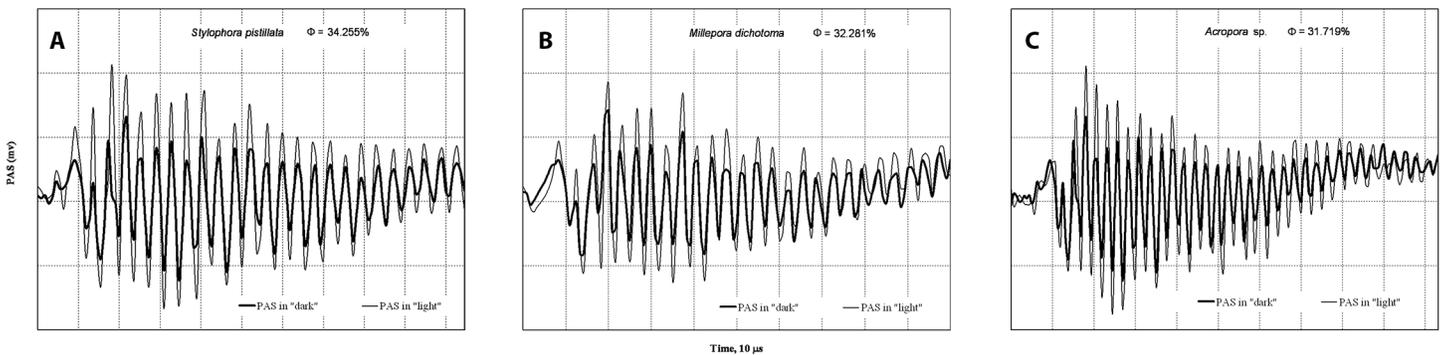


Fig. 4. Photoacoustic signal from 3 different corals' fragments [*Stylophora pistillata* (A), *Millepora dichotoma* (B), *Acropora* sp. (C)], whereas PAS in "dark" is the photoacoustic signal generated by a laser pulse in the dark and PAS in "light" is a signal obtained under saturating continuous light. The area between the two lines is proportional to the fraction of the pulse's light energy stored as products of photosynthesis.

ing a way for future, rapid, nondestructive, field monitoring of bleaching, and eutrophication effects.

The good correlation obtained, regardless of the species-specific differences in polyp size and skeletal architecture, pro-

vides a powerful nondestructive method for following the effects of partial through complete bleaching photoacclimation (Falkowski et al. 1985) and of eutrophication (Dubinsky 1980; Dubinsky et al. 1986).

The photosynthesis versus energy (P versus I) curve based on our photoacoustic method show the progressive closure of “traps” evident from the increase in the ratio of thermally dissipated energy in relation to the complement of the photochemically stored fraction. This curve was obtained by a stepwise increase in the intensity of the continuous background illumination. Hence the shape of the photoacoustically generated photosynthesis to light relationship resembles curves based on oxygen evolution and variable fluorescence (Fig. 2).

These differ from oxygen data in the absence of a parallel to the oxygen uptake by dark respiration and from variable fluorescence by the summation of all pathways leading to the thermal dissipation of harvested light energy.

The exposure of a coral to a stepwise increase in temperature and in time from 24°C to 30°C (Fig. 5), resulted in an immediate concomitant reduction in photosynthetic energy storage efficiency, from $27 \pm 0.5\%$ to $19 \pm 0.5\%$, demonstrating the potential of photoacoustics-based approaches in the monitoring of warming effects on corals, and the detection of thermal-stress sensitive and tolerant coral species and zooxanthellae clades.

The PA-based method allows the non-destructive comparison of various states of energy use by aquatic phototrophs such as zooxanthellate corals and hydrozoans, seaweeds, seagrasses, algal mats, turf and encrusting algae, photoacclimation, eutrophication, and pollution.

Of special timeliness is the convenience of the method for detecting early signs of global climate change related stressors, such as seawater warming and ocean acidification, manifested as decrease in photosynthetic energy storage efficiency.

Comments and recommendations

The clear advantage of the photoacoustics-based determination of photosynthetic energy storage efficiency is its clear dependence on first principles, namely, the immediate conversion of light energy absorbed by the algal layer of corals, but not stored in any products of photosynthesis. It allows the early detection of any decline in the health and performance of the zooxanthellae, which provides the energy basis of coral colonies and entire reefs. It reveals any stressor effect, such as seawater warming, acidification, and pollution.

Based on our results, we recommend the expansion of the method for seaweeds, turf algae, and algal and cyanobacterial mats. Furthermore, a SCUBA-diver-operated instrument is already being developed.

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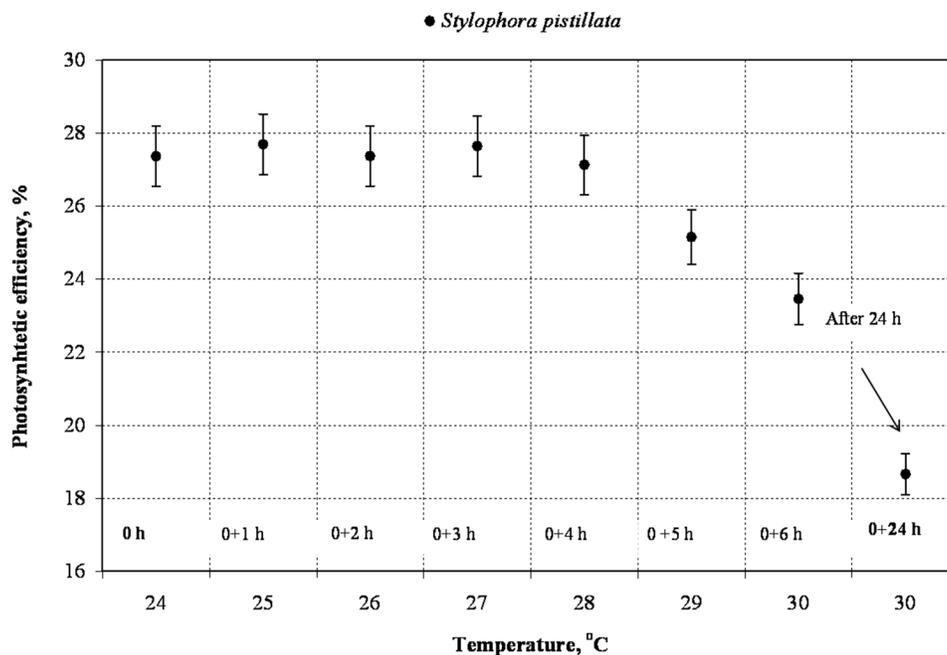


Fig. 5. The deleterious effect of temperature and time increase on photosynthetic efficiency of a zooxanthellate coral as determined by photoacoustics.

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