The Journal of Experimental Biology 209, 273-283
Published by The Company of Biologists 2006
doi:10.1242/jeb.01983

Diel 'tuning' of coral metabolism: physiological responses to light cues

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Accepted 14 November 2005

Summary

Hermatypic–zooxanthellate corals track the diel patterns of the main environmental parameters – temperature, UV and visible light – by acclimation processes that include biochemical responses. The diel course of solar radiation is followed by photosynthesis rates and thereby elicits simultaneous changes in tissue oxygen tension due to the shift in photosynthesis/respiration balance. The recurrent patterns of sunlight are reflected in fluorescence yields, photosynthetic pigment content and activity of the two protective enzymes superoxide dismutase (SOD) and catalase (CAT), enzymes that are among the universal defenses against free radical damage in living tissue. All of these were investigated in three scleractinian corals: *Favia favus*, *Plerogyra sinuosa* and *Goniopora lobata*. The activity of SOD and CAT in the animal host followed the course of solar radiation, increased with the rates of photosynthetic oxygen production and was correlated with a decrease in the maximum quantum yield of photochemistry in Photosystem II (PSII) ($\Delta F'/F_m'$). SOD and CAT activity in the symbiotic algae also exhibited a light intensity correlated pattern, albeit a less pronounced one. The observed rise of the free-radical-scavenger enzymes, with a time scale of minutes to several hours, is an important protective mechanism for the existence and remarkable success of the unique cnidarian–dinoflagellate associations, in which photosynthetic oxygen production takes place within animal cells. This represents a facet of the precarious act of balancing the photosynthetic production of oxygen by the algal symbionts with their destructive action on all living cells, especially those of the animal host.

Key words: SOD, catalase, Photosystem II, zooxanthellae, photosynthesis, *Favia favus*, *Plerogyra sinuosa*, *Goniopora lobata*.

Introduction

The symbiotic association between zooxanthellae and corals responds to the surrounding environment by both fast acclimation and long-term adaptation of each partner in this association. Long-term adaptations involve changes in genotypes and even replacement of the zooxanthellae species (Baker, 2003). Daily changes in light intensity and spectrum, temperature, and nutrient and food availability cause changes in respiration and photosynthesis, enzyme activity and pigment composition and concentration. Increasing irradiance results in an increase in photosynthesis rates (Falkowski and Raven, 1997). Under high irradiance levels in shallow reefs, corals experience elevated oxygen partial pressures (P_{O_2}) in their tissues as a result of the oxygen produced by their zooxanthellae (Dykens and Shick, 1982), apparently without any cellular damage. High light levels, elevated seawater temperature and UV radiation lead to an increase in reactive oxygen species (ROS) within the algae as well as in the host (Lesser and Shick, 1989a,b; Dykens et al., 1992), which is harmful to both (Lesser and Shick, 1989a,b).

Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase function together to inactivate the harmful oxygen free radical and hydrogen peroxide (H_2O_2), thereby preventing subsequent cellular damage (Fridovich, 1986; Asada and Takahashi, 1987). Protective mechanisms against active oxygen species in both the animal host and its zooxanthellae (Lesser and Shick, 1989a,b) have been described. Responses include changes in pigment content and in the activities of SOD, CAT and ascorbate peroxidase (Shick et al., 1995).

The photosynthesis–irradiance relationship in corals changes in response to ambient light (Falkowski et al., 1990). Zooxanthellae, like most algae, photoacclimate to changes in irradiance by adjusting light-harvesting and utilization capability (for reviews, see Falkowski and Owens, 1980; Stambler and Dubinsky, 2004), by changing cellular pigment concentrations (Dubinsky et al., 1984; Porter et al., 1984). Photoacclimation also includes changes in the respiration of the zooxanthellae, the quantum yield and the light-saturated rate of photosynthesis (Porter et al., 1984). Various photoacclimation processes differ in their kinetics (Fisher et

al., 1996). While most of these changes take longer than a day, some, such as polyp expansion and contraction, occur within minutes (Levy et al., 2003). Changes in the density of the zooxanthellae and reshuffling of their species occur within days, whereas changes in growth form of the coral colony may take several months to years (Graus and Macintyre, 1982; Miller, 1994; Todd et al., 2004), and genetic selection takes thousands of years (Falkowski et al., 1990).

In the past few decades, coral reefs have been exposed to natural and anthropogenic stresses, such as increases in temperature and UV radiation, which cause major bleaching events over entire coral reef systems (e.g. Glynn, 1993, 1996; Hoegh-Guldberg, 1999; Fitt et al., 2001; Coles and Brown, 2003; Jokiel, 2004). Eutrophication and pollution are likely to act synergistically with temperature in rendering corals more susceptible to bleaching and disease (Dubinsky and Stambler, 1996). Hypotheses for the mechanism of bleaching include (1) elevated oxygen tension in the symbionts and the host, leading to internal damage by ROS (Shick et al., 1996), (2) reduction of the photosynthesis/respiration ratio, producing an energetic imbalance (Jokiel and Coles, 1990), and (3) free-radical-induced damage to Photosystem II (PSII) of the zooxanthellae (Lesser, 1996), triggering a membrane lipid mediated apoptosis cascade (Tchernov et al., 2004) and in some cases pathogen infection (see Rosenberg and Loya, 2004).

The aim of the present study was to document some responses of corals and their symbionts to the diel patterns of solar radiation. The study of concomitant changes in metabolism, oxygen evolution, fluorescence yields, pigments, and protective enzymes (SOD, CAT) correlated or tracking the course of sunlight, will afford a better understanding of the processes occurring in the host and in the zooxanthellae, allowing corals to optimize the benefits of photosynthesis while minimizing the inevitable risks of concurrent oxidative damage.

Materials and methods

Collection and incubation of colonies

High-light-adapted colonies (Falkowski and Dubinsky, 1981) of *Favia favus* Forskal, *Plerogyra sinuosa* Dana and *Goniopora lobata* Milne Edwards and Haime of similar size (~10 cm diameter) were collected from a depth of 4–5 m. These corals were chosen because we have information on some aspects of their energetics, such as prey capture, photosynthesis and their expansion/contraction behavior (see Levy et al., 2003, 2004, 2005a,b). The daily metabolism (photosynthesis and respiration) and chlorophyll fluorescence measurements were performed during July–August 2001. From December 2001 until March 2002, additional colonies of the same species, ~30 cm in diameter, were collected from 4–5 m-depth next to the Interuniversity Institute for Marine Sciences of Eilat (the H. Steinitz Marine Biology Laboratory), Israel. Three mother colonies from each species were divided into six subcolonies of ~5 cm diameter each and placed at 4–5 m-depth for 75 days for 'healing' and acclimation, before experimentation. One subcolony from each species was placed in the respirometer (see below) for 24-h measurements. After measurement, these subcolonies were frozen in liquid nitrogen. Parallel to the respirometer experiment, every two hours during daytime (08.00–16.00 h) and before dawn (05.00 h), three subcolonies from each species were sampled and immediately frozen in liquid nitrogen. The samples were stored for 1–2 weeks at –70°C before analysis of the biomass parameters and enzyme activity.

Measurement of the light spectrum and UV

The light spectrum was measured at 5 m-depth (in the Gulf of Eilat, Red Sea, in front of the Interuniversity Institute for Marine Science). Measurements were conducted on several cloudless days during 15–19 February 2001. A spectral scan was performed using a Li-Cor LI1800 scanning spectroradiometer (Lincoln, NE, USA). Readings were conducted every 30 min at 2 nm interval spans between 300 and 750 nm (units are $W\,m^{-2}\,nm^{-1}$). Only data from 15 February are presented since that day was considered representative for a cloudless day. The data represent only UV measurements between 300 and 400 nm, which include UVA + UVB.

Photosynthesis and dark respiration

The *in situ* oxygen flux data were obtained using twin, three-chamber submersible respirometers (AIMS, Townsville, QLD, Australia). This instrument is equipped with UV-transparent chambers, each with an oxygen sensor (Kent EIL galvanic type ABB, Stonehouse, Gloucestershire, UK), one light meter (Li-Cor 4π underwater quantum sensor), a temperature probe and a data logger. A centrifugal pump flushes the water in the chambers at programmable intervals. Twenty-minute intervals were used for these experiments (Fabricius and Klumpp, 1995). Prior to the incubation period, the colony surfaces were carefully cleaned of epiphytes and other debris using a small toothbrush. The respirometers were deployed at 4–5 m-depth in front of the H. Steinitz Marine Biology Laboratory (near the incubation site). Data processing was performed using the AIMS 'Respiro' program for calibrating and normalizing the data. Respiration was measured as oxygen uptake in the dark. Parameters for the photosynthesis (*P*) *versus* energy (*E*) curves were calculated from a non-linear curve-fitting, including α (initial slope). P_{max} (light-saturated photosynthesis rate and compensation light levels), E_{opt} (optimum irradiance), E_{com} (compensation intensity) and E_k (saturating intensity) were calculated from a non-linear curve-fitting based on theoretical models of a hyperbolic tangent equation (Ben-Zion and Dubinsky, 1988).

FRR fluorescence measurements of the quantum

Diel changes in chlorophyll fluorescence and fluorescence characteristics of the three symbiotic corals were monitored for three consecutive days for a diurnal cycle from 31 July to 2 August 2001, using the SCUBA-based Fast Repeating Rate

Fluorometer (FRRF; Gorbunov et al., 2000). The FRRF measures the chlorophyll fluorescence yield using a sequence of flashlets that gradually close the PSII reaction centers, leading to an increase in chlorophyll fluorescence (Kolber and Falkowski, 1993). The maximum quantum yield of photochemistry in PSII was determined in a dark-adapted state as the ratio of $F_v/F_m=(F_m–F_o)/F_m$, according to Butler (1972), where F_v is variable fluorescence, F_m and F_o are maximum and minimum yields of chlorophyll *a* fluorescence measured in a dark-adapted state (relative units). The steady-state quantum yield was measured under illumination by ambient light and defined by $\Delta F'/F_m'=(F_m'–F')/F_m'$ (see Gorbunov et al., 1999, 2000; Lesser and Gorbunov, 2001). The quantum yield of irradiance-stimulated thermal dissipation [i.e. non-photochemical quenching (NPQ)] is assessed from the ratio of $(F_m–F_m')/F_m$ (Gorbunov et al., 2001). The instrument was placed on a tripod at 5 m-depth, and measurements were taken automatically from the top part of the coral colony (Lesser and Gorbunov, 2001) at 10-min intervals. Cross section was calculated (Gorbunov et al., 2001). The data presented show mean measurements for each hour.

Biomass parameters

Tissue homogenates were prepared by removing all tissue by an airbrush method, which is similar to the Water-Pik method (Johannes and Wiebe, 1970) but uses airjet to strip the tissue of the coral skeleton into a few ml of 100 mmol l^{-1} phosphate buffer (pH 7.0). The volume of the homogenate was measured and subsamples taken for determination of: (1) zooxanthellae density, from direct counts on a Neubauer hemacytometer; (2) chlorophyll *a* concentration, measured spectrophotometrically on a Cary spectrophotometer (Varian, Palo Alto, CA, USA) in 90% acetone (Jeffrey and Humphrey, 1975) and (3) enzyme activity, quantified using the methods described below. The surface area was calculated based on a digital photograph using the program Image tool (Uthscsa Image Tool for Windows, v. 2.00, University of Texas, San Antonio, TX, USA).

Enzyme assays

The homogenate was centrifuged twice at 1500 ***g*** for 15 min. The supernatant was used for analysis of animal protein and enzyme activity. The pellets containing the zooxanthellae were suspended in 2 ml 100 mmol l^{-1} phosphate buffer (pH 7.0). One milliliter was taken for HPLC analysis, and an additional 1 ml was dissolved by sonication (Misonix 3000; 4×15 s pulses at 35 W). The sonicated suspension was replaced by a 0.05% Triton X-100 solution in 1 ml 100 mmol l^{-1} phosphate buffer (pH 7.0). After incubation for 10 min, the suspension was centrifuged at 14 000 ***g*** for 30 min and used for protein and enzyme analyses. The enzymes were examined in the cytosoluble fraction of protein extractions. SOD activity was assayed spectrophotometrically as described by Elstner and Heupel (1976) and Oyanagui (1984). Standards were prepared using bovine erythrocyte SOD (Sigma) for each set of samples. Catalase activity was assayed spectrophotometrically by monitoring H_2O_2 depletion at 240 nm. All assays were conducted at 25°C and expressed as enzyme activity units (U) per mg protein. SOD and CAT activities were averaged and presented as a percentage of the total maximum activity. Protein content was determined by the Bradford assay (Bradford, 1976).

Pigment analysis (HPLC)

For pigment analysis, frozen subsamples were extracted in 1 ml cold 100% acetone, vortexed and centrifuged. The supernatant was transferred to a test tube and kept in the dark at 4°C for approximately 1 h. The acetone extract was then filtered onto a GF/F filter, and 0.3 ml 1 mol l^{-1} ammonium acetate was added in order to facilitate the separation of the hydrophilic components of the extract (Zapata et al., 1987). The reverse-phase HPLC system consisted of a multiple solvent delivery pump (CM4000 LDC–Milton-Roy, Riviera Beach, FL, USA), an injector (Rheodyne) equipped with a 100 μl loop and a C-18, 25×4.6 mm (Alltech, Deerfield, IL, USA) Spherisorb column. The pigments were detected with a variable-wavelength spectrophotometer (LDC–Milton-Roy) set at 436 nm. Data were recorded and processed by a digital-analog converter and software (Jasco-Borwin, La Fontanil, France). Two solvents were used in the system: solvent A consisted of 30% 1 mol l^{-1} ammonium acetate (Sigma) in double-distilled deionized water and 70% methanol (HPLC grade; Bio Lab, Jerusalem, Israel); solvent B consisted of 30% ethylacetate (HPLC grade, Bio Lab, Israel) and 70% methanol. The solvent program exhibited a linear increase in solvent B from 20 to 60% in 7 min, a plateau at 60% for 5 min, a linear increase in solvent B from 60 to 100% from 12 to 20 min and was then maintained at 100% solvent B for 20 min.

Pigment identification was facilitated by the use of ChromaScope (BarSpec, Rehovot, Israel), a spectral peak analyzer that allows scanning of pigments separated by the HPLC system in the range of 360–700 nm. The spectral data of the separated peaks and their retention times were used as the parameters for peak identification using published data (Rowan, 1989; Jeffrey et al., 1997) and were compared to standards (Yacobi et al., 1996). The major pigments found in this study were isolated online; their concentrations were determined and subsequently used for quantification. Quantification of the chromatograms was facilitated by injection of known pigment concentrations into the HPLC system and calculation of the response factor based on the area under the peak. All pigment concentration calculations are presented as the mean of duplicate measurements. Individual measurements did not differ by more than 10%. The pigment data collected by HPLC were expressed in relation to moles of chlorophyll *a* (Chl *a*).

Statistical analysis

Differences between SOD and CAT activities were estimated using one-way analysis of variance (ANOVA) and the Student–Newman–Keuls (SNK) test at the 5% significance level. The means of enzyme activity for each coral species

were converted using the arcsine function. The Pearson correlation factor was used in order to determine the relationship between light intensity and enzyme activity (Sokal and Rohlf, 1981). Values are represented as means ± s.d.

Results

Metabolism and photosynthesis

Light intensity at the 4–5 m-depth water on the reef in Eilat (Gulf of Aqaba) on 3–5 March 2002 reached a maximum of 1100 μmol quanta m^{-2} s^{-1} (Fig. 1A), while in July–August it reached a value of ~2000 μmol quanta m^{-2} s^{-1}. The intensity values of UVA + UVB radiation in February 2001 at the 5-m depth is presented in Fig. 1B. The net oxygen evolution rate tracked the diurnal course of solar energy flux (Fig. 1A), while respiration rates did not change significantly during the night. Typical photosynthesis *versus* irradiation curves for colonies of *Favia favus*, *Plerogyra sinuosa* and *Goniopora lobata* are presented in Fig. 1C–E. The optimum light intensity (E_{opt}) at which photosynthesis is at a maximum without exhibiting photoinhibition was similar for all three species, approximately 1100 μmol quanta m^{-2} s^{-1}, which is close to the maximum radiation intensity at this depth. The compensation point (E_{com}) at which photosynthesis and respiration rates are equal was reached at approximately 10–15% of the optimum light intensity, between 119 and 155 μmol quanta m^{-2} s^{-1}. *Favia favus* and *P. sinuosa* were light-saturated near 700 μmol quanta m^{-2} s^{-1}, while *G. lobata* reached almost 950 μmol quanta m^{-2} s^{-1}. The maximum photosynthesis rate of *F. favus* colonies (0.11±0.03 μmol O_2 cm^{-2} min^{-1}) was lower than that of both *G. lobata* and *P. sinuosa* (0.22±0.05 and 0.22±0.13 μmol O_2 cm^{-2} min^{-1}, respectively). The highest P_g/R_{24h} ratio was found in *F. favus* (4.37±0.68), while *P. sinuosa* (3.78±0.18) and *G. lobata* (3.48±0.23) exhibited lower values (one-way ANOVA; $P<0.05$; Table 1). Net oxygen production followed ambient light intensity at the same depth of 5 m, and the peak of oxygen evolution was reached at midday. Photoinhibition was not observed even at noon (Fig. 2).

FRR fluorescence

In situ measurements of chlorophyll fluorescence yields revealed a diel variation in response to ambient illumination. The variable fluorescence $\Delta F'/F_m'$ declined from ~0.40 (arbitrary units, a.u.) in the early morning to ~0.07 at midday for *P. sinuosa*, ~0.25 for *F. favus* and ~0.1 for *G. lobata* (Fig. 2). Decreases in $\Delta F'/F_m'$ values were found during the midday hours (between 10.00 and 14.00 h) in *F. favus* and *G. lobata* (Fig. 2A,C), while in *P. sinuosa* the values were low until late afternoon (16.00 h; Fig. 2B). In all three species from early

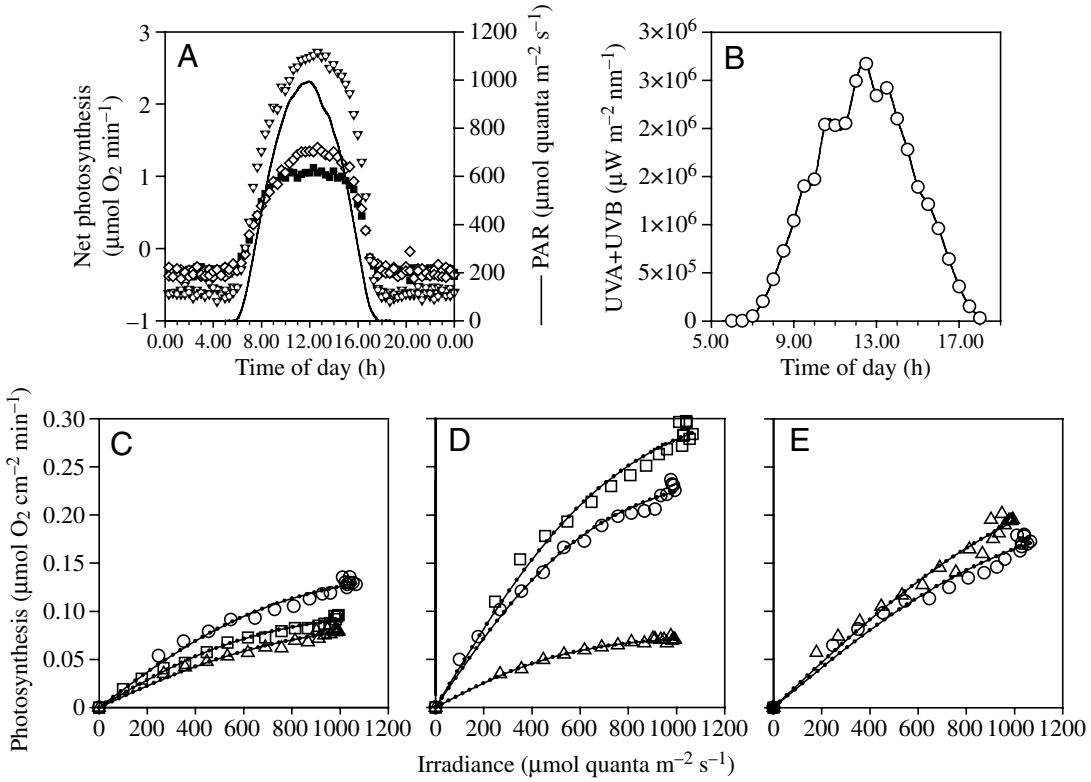

Fig. 1. (A) Changes in light intensity at a depth of 4–5 m in Eilat, Red Sea on 3 March 2002 (solid line) and net oxygen fluxes measured using the three-chamber submersible respirometer. The values are per liter and disregard differences in coral size. *G. lobata* (filled squares), *F. favus* (triangles) and *P. sinuosa* (diamonds). (B) UVA + UVB radiation (300–398 nm) at the 5-m depth observed from diurnal measurements conducted in February 2001. (C–E) *In situ* gross photosynthesis–irradiance curves for the corals (C) *Favia favus* (N=3), (D) *Plerogyra sinuosa* coral (N=3) and (E) *Goniopora lobata* (N=2). Measurements were performed in the Red Sea, Eilat on three consecutive days, 3–5 March 2002.

Table 1. *Means of the photosynthetic (P vs E) values and related parameters for the three coral species*

	F. favus (*N*=3)	*P. sinuosa* (*N*=3)	*G. lobata* (*N*=2)
α (μmol O_2 cm^{-2} min^{-1}) (μmol quanta m^{-2} s^{-1})$^{-1}$	$1.50\times10^{-4}\pm3.64\times10^{-5}$	$2.50\times10^{-4}\pm1.67\times10^{-4}$	$2.23\times10^{-4}\pm1.83\times10^{-5}$
E_{com} (μmol quanta m^{-2} s^{-1})	118.75±6.57	130.30±21.08	153.96±140.43
E_k (μmol quanta m^{-2} s^{-1})	752.37±51.63	677.90±101.51	952.95±130
E_{opt} (μmol quanta m^{-2} s^{-1})	1017.30±40.36	1017.30±40.36	1025±53.67
P_{max} (μmol O_2 cm^{-2} min^{-1})	0.11±0.03	0.22±0.13	0.22±0.05
Respiration (oxygen flux) (μmol O_2 min^{-1})	–1.7±0.2	–2.3±0.35	–1.4±0.28
P_g/R_{24h}	4.37±0.68	3.78±0.18	3.48±0.23
r^2	0.98±0.01	0.99±0.004	0.98±0.004

The values are means ± s.d. and were constructed from in situ measurements performed using the submersible respirometer.

Abbreviations: α, initial slope; E_{com}, compensation intensity; E_k, saturating intensity; E_{opt}, optimum irradiance; P_{max}, light-saturated photosynthesis rate and compensation light levels; P_g/R_{24h}, rate of gross photosynthesis to dark respiration integrated over 24 h.

afternoon, there was an increase in $\Delta F'/F_m'$ values as light intensity decreased. In all three species, $\Delta F'/F_m'$ values were inversely correlated to the net oxygen production (Fig. 2A–C). The functional absorption cross section of PSII (σ_{PSII}') decreased slowly with increasing irradiance, with minimum values being observed between 11.00 and 14.00 h in all coral species examined (Fig. 3; data not presented for *G. lobata*). The recovery in σ_{PSII}' occurred in late afternoon, when light intensity decreased down to less than ~500 μmol quanta m^{-2} s^{-1} (Fig. 3). The σ_{PSII}' values did not change significantly during the night and remained constant (data not presented). The irradiance-induced decrease in $\Delta F'/F_m'$ was accompanied by an increase in the quantum yield of NPQ in all corals (Figs 2, 3). In *F. favus* (Fig. 2A) and *G. lobata* (not presented), $\Delta F'/F_m'$ decreased to a level of ~0.1–0.25 at a maximum light intensity of 2000 μmol quanta m^{-2} s^{-1}, while in *P. sinuosa* $\Delta F'/F_m'$ values were less than ~0.1 (Fig. 2B). In *F. favus*, NPQ maximum values reached ~0.4, whereas *P. sinuosa* exhibited higher NPQ values of ~0.65 when light intensity was above ~1800 μmol quanta m^{-2} s^{-1} (Fig. 3).

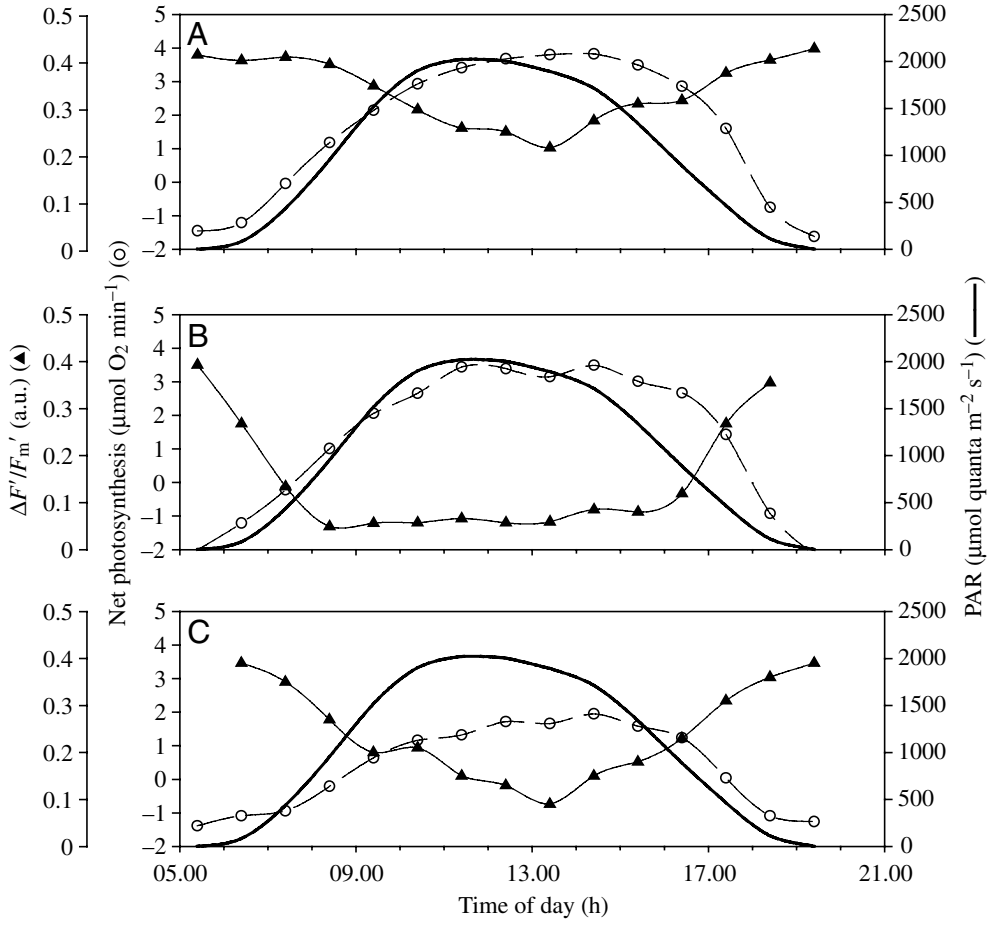

Fig. 2. Diel cycles of *in situ* measurements of net photosynthesis (circles) and light intensity (solid line) of the corals *F. favus* (A), *P. sinuosa* (B) and *G. lobata* (C), performed using the three-chamber submersible respirometer. Changes in the maximum quantum yield of photochemistry in PSII under ambient light ($\Delta F'/F_m'$; triangles) were determined using the fast repetition rate fluorometer (FRRF). Measurements were performed in Eilat, Red Sea between 31 July and 2 August 2001.

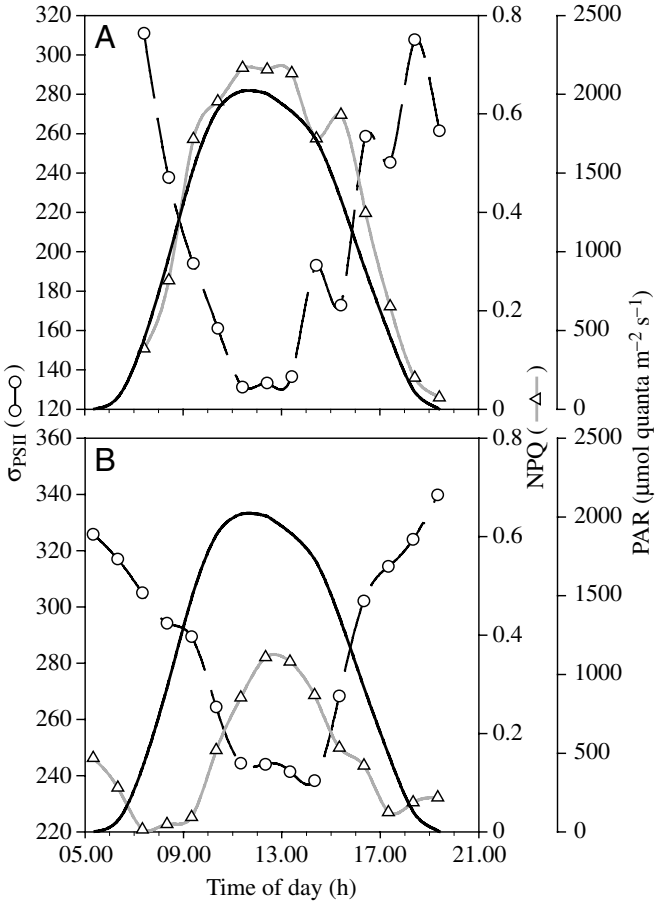

Fig. 3. Diurnal cycle of the functional absorption cross section for PSII (σ_{PSII}; circles), non-photochemical quenching (NPQ) (triangles) and light intensity (solid line) as recorded in the corals (A) *P. sinuosa* and (B) *F. favus*.

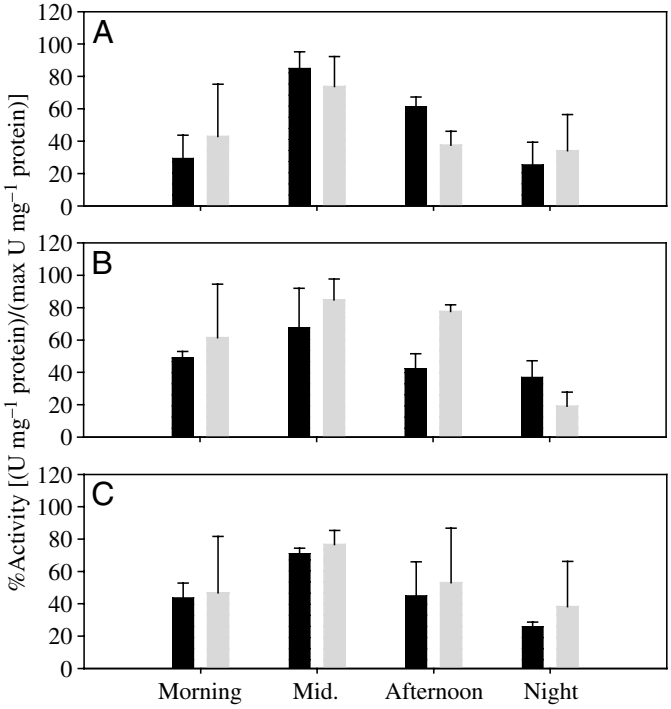

Fig. 4. Diel cycle of superoxide dismutase (SOD) enzyme activities from animal tissue and symbiotic zooxanthellae collected at the 5-m depth in Eilat, Red Sea. Measurements were performed between 3 and 5 March 2002. Activity is presented as % activity [(U mg^{-1} protein)/(max U mg^{-1}protein)] for (A) *F. favus* (*N*=3), (B) *G. lobata* (*N*=2) and (C) *P. sinuosa* (*N*=3). The black bars represent SOD activity of the animal tissue and the gray bars represent SOD activity of the zooxanthellae. Values are represented as means ± s.d. (night=05.00 h; morning=08.00 h; mid.=10.00–14.00 h; afternoon= 16.00 h).

Enzyme activity

Superoxide dismutase (SOD) activity in the animal tissue of the three species exhibited a similar trend (Fig. 4) of increasing activity with irradiance and net photosynthesis productivity (one-way ANOVA for *F. favus*, $P<0.0001$; for *G. lobata* $P<0.05$; for *P. sinuosa* $P<0.001$). In tissues of *P. sinuosa* and *F. favus*, SOD activity values observed between 10.00 and 14.00 h were significantly higher than during the rest of the day and night (SNK, $P=0.006$ and $P=0.009$, respectively). SOD activity in *G. lobata* coral tissue was significantly higher only in the afternoon (16.00 h) and during the night (05.00 h) (SNK, $P<0.05$; Fig. 4B). SOD activity in the isolated zooxanthellae of the three coral species did not differ significantly during day or night (one-way ANOVA, $P>0.05$), although a clear trend of increasing activity with increased light intensity could be observed (Fig. 4A–C).

Catalase (CAT) activity revealed a similar pattern, i.e. increased activity in the coral tissue with increasing irradiance and oxygen evolution (Fig. 5A–C). The highest value was observed during midday in *F. favus* (SNK, $P<0.0001$) and in *G. lobata* (SNK, $P<0.05$), while in *P. sinuosa* the highest activity values were observed in the morning and midday. The morning values were significantly higher than night-time/dawn values (SNK, $P=0.034$). CAT activity in the symbiotic zooxanthellae of *P. sinuosa* peaked during the morning hours (Fig. 5C) compared with midday and night-time (SNK, $P=0.037$). Although light intensity and CAT activity were correlated in *F. favus* zooxanthellae, the results were not significant ($P=0.07$; Fig. 5A). *G. lobata* did not reveal a significant increase in CAT activity in any of the measurements performed in this study ($P=0.34$). Mean diel values of SOD and CAT activity in the host and in the zooxanthellae per species are presented in Table 2. A high correlation was found between SOD activity and light intensity in both the animal tissue and algal cells (Table 2). A correlation was found between CAT activity and light intensity in the tissue of *F. favus* (Pearson's correlation, $r=0.811$) and *G. lobata* ($r=0.969$), while *P. sinuosa* exhibited a low correlation ($r=0.594$). CAT activity of the zooxanthellae exhibited a high correlation with light intensity only in *F. favus* ($r=0.951$), whereas in *G. lobata* ($r=0.441$) and *P. sinuosa* ($r=-0.055$) (Table 2), the correlation was low.

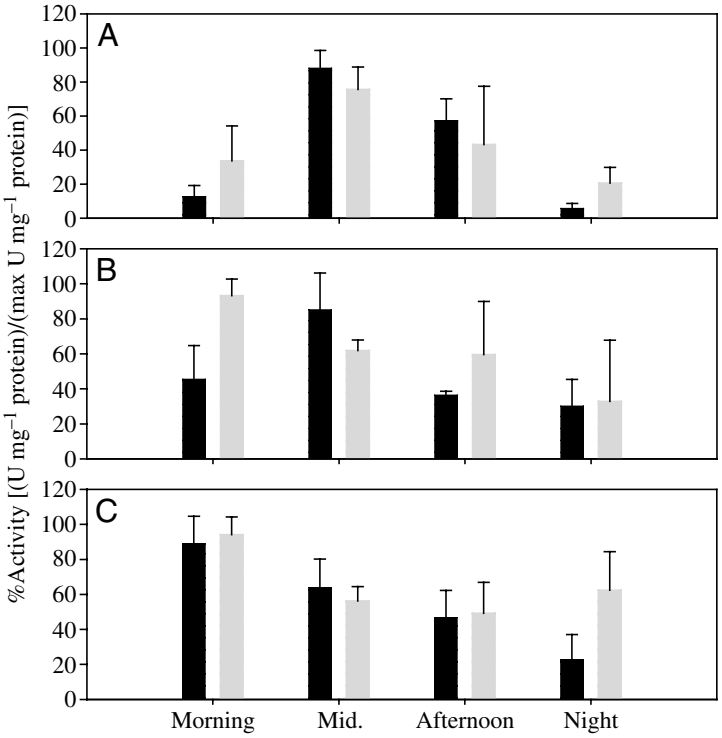

Fig. 5. Diel cycle of catalase (CAT) activity from coral tissue and symbiotic zooxanthellae at the 5-m depth in Eilat, Red Sea. Measurements were performed between 3 and 5 March 2002. Activity is presented as % activity [(U mg^{-1} protein)/(max U mg^{-1}protein)] for (A) *F. favus* (*N*=3), (B) *G. lobata* (*N*=2) and (C) *P. sinuosa* (*N*=3). The black bars represent CAT activity of the animal tissue and the gray bars represent CAT activity of the zooxanthellae. Values are represented as means ± s.d. (night=05.00 h; morning=08.00 h; mid.=10.00–14.00 h; afternoon= 16.00 h).

Pigment analysis

The pigment suite of zooxanthellae revealed in the HPLC chromatograms demonstrated the typical components of dinoflagellates (Table 3). The major pigments were Chl c_2, peridinin, dinoxanthin, diadinoxanthin (DD), Chl *a* and β-carotene. Chlorophyllide *a* (Chld *a*) was present in all chromatograms, and its concentrations were pooled with those of Chl *a*. Even though the samples were preserved in liquid N_2 immediately after collection of the coral, we were not able to detect diatoxanthin, which is a signature pigment for the photoprotective response. It is known that this pigment vanishes within minutes if algal cells are allowed to relax in low light (Goericke and Welschmeyer, 1992). The statistical analyses did not detect any significant differences (one-way between pigment compositions ANOVA, $P>0.05$) (Table 3). The three coral species were almost uniform in their pigment concentration relative to Chl *a* throughout the day (Table 3).

Discussion

Diel changes in response to the course of sunlight are among the most interesting phenomena of coral physiology. This includes patterns of calcification and photoacclimation. The latter comprises changes in photosynthesis and respiration rates, P_g/R_{24h} as well as pigment content (reviewed by Falkowski et al., 1990; Barnes and Chalker, 1990). Changes in enzymatic activity and behavioral responses, such as variations in tentacle expansion and contraction (Levy et al., 2003), are all light dependent and have been studied for many years (Brown et al., 2002a; Stambler and Dubinsky, 2004). Hoegh-Guldberg and Jones (1999), Lesser and Gorbunov (2001) and Gorbunov et al. (2001) monitored diurnal *in situ* chlorophyll fluorescence yields. Brown et al. (1999) followed the diurnal changes in photochemical efficiency and xanthophyll concentrations.

The present study highlights the dynamics of the diurnal cycle of corals *in situ* and their mechanism of continuous 'tuning' in relation to the course of light and UV radiation and their penetration into shallow water. The three corals studied, *Favia favus*, *Plerogyra sinuosa* and *Goniopora lobata*, are common reef-building corals in the Red Sea, from the surface to a depth of 30 m. The distributed three species exhibit a diurnal course in oxygen evolution parallel to that of light intensity. Different P_g/R_{24h} ratios were shown to occur as a result of polyp expansion/contraction, as described by Fabricius and Klumpp (1995) for soft corals. Their data reveal that colonies with expanded tentacles have higher P_g/R_{24h} values (1.3±0.02) than colonies with contracted tentacles

Table 2. *Mean diel activity of superoxide dismutase (SOD) and catalase (CAT) in the host and in the zooxanthellae, Pearson correlation values (r) between light intensity and enzyme activity*

	Enzyme activity		*r*	
	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	SOD	CAT
F. favus animal tissue	6.27±0.8 (18)	27.48±5.0 (18)	0.813	0.811
F. favus zooxanthellae	13.89±3.57 (18)	20.01±8.45 (18)	0.956	0.951
G. lobata animal tissue	1.38±0.18 (12)	11.36±1.75 (12)	0.987	0.969
G. lobata zooxanthellae	7.32±1.1 (12)	3.87±0.73 (12)	0.808	0.441
P. sinuosa animal tissue	1.88±0.23 (18)	5.9±1.02 (18)	0.986	0.594
P. sinuosa zooxanthellae	3.63±0.52 (18)	3.17±0.4 (18)	0.952	–0.0055

Values are means ± s.d. (*N*).

Table 3. *Pigment compositions from the three coral species relative to Chl a at different times of the day*

Pigment/Chl *a* (mol mol^{-1})	*Favia favus*				*Goniopora lobata*				*Plerogyra sinuosa*			
	05.00 h	08.00 h	10.00–14.00 h	16.00 h	05.00 h	08.00 h	10.00–14.00 h	16.00 h	05.00 h	08.00 h	10.00–14.00 h	16.00 h
Chld *a*	0.31±0.20	0.38±0.35	0.34±0.17	0.73±0.24	0.22±0.26	0.11±0.09	0.45±0.39	0.31±0.31	0.45±0.55	0.10±0.12	0.24±0.14	0.11±0.07
Chl c_2	0.22±0.04	0.31±0.12	0.26±0.06	0.32±0.06	0.20±0.05	0.29±0.05	0.28±0.08	0.24±0.05	0.28±0.13	0.18±0.10	0.24±0.03	0.23±0.05
Peridinin	0.53±0.10	0.75±0.31	0.74±0.26	1.01±0.98	0.39±0.13	0.97±0.14	0.63±0.10	0.64±0.20	0.86±0.42	0.51±0.33	0.74±0.19	0.63±0.05
Dinoxanthin	0.04±0.01	0.05±0.01	0.04±0.01	0.06±0.01	0.03±0.01	0.04±0.00	0.04±0.02	0.04±0.01	0.03±0.02	0.06±0.06	0.04±0.01	0.04±0.01
Diadinoxanthin	0.29±0.07	0.36±0.09	0.28±0.07	0.43±0.09	0.26±0.02	0.25±0.02	0.31±0.10	0.27±0.01	0.27±0.09	0.18±0.05	0.27±0.06	0.26±0.07
β-carotene	0.04±0.01	0.04±0.01	0.02±0.02	0.04±0.03	0.05±0.00	0.02±0.00	0.04±0.02	0.02±0.03	0.02±0.02	0.03±0.02	0.03±0.02	0.04±0.00

Values are means ± s.d. (N=3).

(1.14±0.02). The P_g/R_{24h} ratios presented in Table 1 for the three species range from 3.48 to 4.37. Generally, $P_g/R_{24(h)}$ ratios in hard corals range between 2 and 4. Scleractinian corals have much higher photosynthetic rates than soft corals (Fabricius and Klump, 1995), which may be related to a far higher density of zooxanthellae cell in host tissue in scleractinians (usually in the range of $0.5–1\times10^6$ zooxanthellae cm^{-2}) than in most octocorallians (with the possible exception of *Tubipora musica*), which is reflected in the higher host respiration/symbiont photosynthesis ratio. The stony corals *F. favus* and *P. sinuosa* expand their tentacles nocturnally and remain open until sunrise, whereas *G. lobata* remains expanded continuously (Levy et al., 2003). *P. sinuosa* can also expand and contract its photosynthetic vesicles in response to varying light intensities, as described by Vareschi and Fricke (1986). Therefore, the different ratios of oxygen evolution and respiration observed by us are likely to reflect polyp behavior such as expansion or contraction of the tentacles (Levy et al., 2003).

Differences in metabolism, especially in respiration rate, can also be influenced by feeding behavior. Porter (1976) suggested that corals with large polyps depend on heterotrophy more than those with small polyps (see Lesser et al., 2000); however, no support for this hypothesis was found in subsequent studies (Haramati et al., 1997). *Goniopora lobata* polyps are small (~5 mm) compared with those of *F. favus* (~11 mm) and *P. sinuosa* (up to ~20 mm). However, *G. lobata* has a greater tentacular surface for feeding than is found in other small polyp corals (Sebens et al., 1997). From the $P_g/R_{24(h)}$ ratios measured, it seems that *G. lobata* exhibits a lower ratio with a small polyp size. This can be related to the changes in tentacle state. The tentacles can be expanded or contracted during daytime, which can influence photosynthetic rate and reduce the ratio of P_g/R_{24h} (Levy et al., 2005a). *Plerogyra sinuosa*, with the largest polyp size, had similar P_g/R_{24h} values (Table 1) to those of *G. lobata*. This results from daytime expansion of the photosynthetic vesicles.

Respirometer data on coral metabolism in shallow waters do not indicate any photoinhibition during the midday hours of July–August 2001 (Fig. 2A–C) and March 2002 (Fig. 1A–E). A similar finding was noted by Goiran et al. (1996) for the symbiotic coral *Galaxea fascicularis* and by Muller-Parker (1984) for the sea anemone *Aiptasia pulchella.* Absence of photoinhibition in both intact coral colonies and whole reefs is common and predictable: while branch tips and surface zooxanthellae are exposed to the full, measured irradiance, most of the symbionts in branched colonies and deep-seated algae in massive species are still light limited. The result is that photoinhibition of high-light-exposed cells is masked by the status of most of the zooxanthellae, a situation akin to that of entire reefs, where surfaces may be photoinhibited, while light in reef crevices and shaded parts of colonies never reaches saturation levels.

Despite the fact that oxygen measurements did not show photoinhibition, the FRRF measurements demonstrate a clear pattern of midday depression in $\Delta F'/F_m'$ associated with increasing light intensity accompanied by an increase in non-

photochemical quenching. As ambient light intensity increased, both steady-state (F') and maximum (F_m') fluorescence yields decreased due to non-photochemical quenching (NPQ) of chlorophyll fluorescence (Lesser and Gorbunov, 2001; Levy et al., 2004). Again, this difference between the data integrated from whole colonies, measured by the respirometer, and those of the FRRF, which observes only a small section of the colony at its exposed surface, is to be expected and underscores the benefits inherent in combining both methodologies concomitantly. NPQ is one of the protective mechanisms against oxidative damage that is associated with the ability to dissipate excess energy as heat (Falkowski and Raven, 1997). The heat from the NPQ process is partly dissipated *via* the xanthophyll cycle, in which diadinoxanthin is converted into diatoxanthin; in this way, corals can keep excess light energy from damaging components of the photosynthetic pathway (Ambarsari et al., 1997; Brown et al., 1999, 2002b). The diurnal changes in the chlorophyll fluorescence cycle (Fig. 2) are similar to those obtained in earlier studies by Brown et al. (1999), Hoegh-Guldberg and Jones (1999), Lesser and Gorbunov (2001) and Gorbunov et al. (2001). The higher values of NPQ found in *P. sinuosa* as compared with *F. favus* (Fig. 3) indicate that this species is capable of dissipating excess excitation energy more efficiently through the xanthophyll cycle, and by doing so, this coral might be less prone to bleaching events (see Warner et al., 1999). Diel periodicity may lead to differences in the pigment concentrations due to circadian rhythm, as reported for phytoplankton (Marra, 1980; Owens et al., 1980; Post et al., 1984). In the present study, photosynthetic pigments did not exhibit temporal patterns in concentration (Table 3). Again, it may well be that such changes did take place but went unnoticed because of the different exposure of the zooxanthellae to light on a mm-to-cm scale, which could not be resolved by us.

High oxygen levels within the host tissues of symbiotic cnidarians are a result of the high rate of oxygen production in zooxanthellae exposed to high irradiance (Lesser et al., 1989a,b). Photosynthetic oxygen evolution by the zooxanthellae *in hospice* can raise tissue oxygen concentrations to $\geqslant$200% saturation (Dykens and Shick, 1982; Kuhl et al., 1995; De Beer et al., 2000). Such levels of photosynthetically produced molecular oxygen act synergistically with sunlight, especially UV radiation, in the presence of photosensitizing agents (e.g. flavins and chlorophylls) to produce active harmful forms of oxygen (Foote, 1976; Asada and Takahashi, 1987). Increased SOD and CAT activity is associated with increased light and UV intensity (Fig. 3; Table 2), suggesting higher O_2^- radical and H_2O_2 production (Levy et al., 2005b). Therefore, a diurnal change in SOD/CAT activity in the host corresponds to the diurnal patterns of solar radiation both in the visible and the UV domains, acting as a protective mechanism against oxygen radicals. It is likely that the animal host is more susceptible to oxidative damage than a plant, where high oxygen tension is the norm; indeed, SOD activity in the symbiotic algae did not show a significant diurnal pattern, although it did correlate with light intensity (Figs 3, 4; Table 2). The fact that SOD/CAT activity was higher in the morning suggests that, as light increases ahead of any photoacclimation processes, the algae are susceptible at that time to the increasing irradiance and UV penetration. Oxidative stress and apoptosis occur in both the animal and the zooxanthellae (Dunn et al., 2002; Lesser and Farrell, 2004). However, we still believe that oxidative stress is primarily an animal response, aggravated by the presence of symbiotic algae that can cause hyperoxia (Nii and Muscatine, 1997). The work done by Nii and Muscatine (1997) on sea anemones suggested that exposure to chronic stress may cause symbionts to release oxidants directly and that oxidative stress in the sea anemone *A. pulchella* is primarily an animal response. Downs et al. (2002) proposed that algae-generated H_2O_2 could diffuse from the algal symbionts into the coral cytoplasm. Once inside the coral cytosol, H_2O_2 can be neutralized by protective enzymes or converted into the hydroxyl radical, which can eventually lead to coral bleaching (Levy et al., 2005b). Therefore, the rise in free-radical-scavenger enzymes in the host is likely to be a defense reaction to the accumulation of free radicals within the host tissue. The difference in enzymatic activities between species indicates that although the acclimation process depends on environmental conditions, genetic variation among species controls the function of these enzymes and, thus, plays a role in the survival and the depth distribution of different corals in the coral reef.

Excessively high photosynthetically available radiation (PAR) is potentially detrimental to photosynthetic organisms. The photosynthetic apparatus saturates rapidly at relatively low flux levels (Hoegh-Guldberg and Jones, 1999). Above these levels, light energy damages the photosynthetic machinery (Walker, 1992; Foyer et al., 1994; Osmond, 1994). Short wavelengths (290–400 nm) and elevated temperature may act synergistically with intense radiation, causing destructive effects in both partners (host and symbiotic algae; Jokiel, 1980; Shick et al., 1996). These environmental stresses may lead to photoinhibition, a reduction in photosynthesis and an increase in respiration, and to a change in pigment content. The outcome of these environmental effects leads to the evolution of several protective mechanisms. These include the production of mycosporine-like amino acids for which a UV-absorbing function has been inferred (Dunlap and Chalker, 1986; Lesser and Shick, 1989b; Lesser, 2000; Shick and Dunlap, 2002) and the development of active oxygen scavenging systems (Shick et al., 1996). The present study indicates that shallow water corals respond rapidly to the prevailing internal and external conditions and accomplish fine-tuned adjustments to ensure that they continue to function effectively.

In this fine-tuning, the coral host plays the role of an important guardian, as can be observed from the increase in the activity of two free-radical scavenging enzymes, SOD and CAT. The adjustments occur within a few minutes to several hours, while changes in the pigment content may occur on a scale of days. In addition, increasing NPQ while reducing the cross section of PSII are important mechanisms, protecting both algae and host from harmful excess energy. We suggest that the symbiotic dinoflagellates within the host live in an environment that protects them from chronic photoinhibition (Gorbunov et

al., 2001). This symbiotic association owes its success partially to the effectiveness of the host's anti-oxidative defenses and partially to the photoacclimative plasticity of the zooxanthellae. Since corals have evolved in stable tropical environments, we assume that even slight changes in the environment, such as rising sea-surface temperatures, increased UV load, lowering of ambient pH and bacterial infection, occurring on time scales too short to allow the necessary genetic adaptation and extending outside the phenotypic ranges covered by acclimation, can upset this unique system.

We thank the staff of the Interuniversity Institute of Marine Sciences in Eilat for their support during our fieldwork. We acknowledge the help of E. Perel, N. Schwalb, M. Ohavia and A. Rivlin for technical assistance, E. Levin for helping in the fieldwork, M. Ben-Zion for helping us with computer programming, and D. Iluz for helping us with light measurements. We also thank A. Perelman for his suggestions on the enzyme work.

References

Ambarsari, I., Brown, B. E., Barlow, R. G., Britton, G. and Cummings, D. (1997). Fluctuations in algal chlorophyll and carotenoid pigments during solar bleaching in the coral *Goniastrea aspera* at Phuket, Thailand. *Mar. Ecol. Prog. Ser.* **159**, 303-307.

Asada, K. and Takahashi, M. (1987). Production and scavenging of active oxygen in photosynthesis. In *Photoinhibition* (ed. D. J. Kyle, C. B. Osmond and C. J. Arntzen), pp. 228-287. Amsterdam: Elsevier.

Baker, A. C. (2003). Flexibility and specificity in coral-algal symbiosis: Diversity, ecology, and biogeography of *Symbiodinium*. *Annu. Rev. Ecol. Evol. Syst.* **34**, 661-689.

Barnes, D. J. and Chalker, B. E. (1990). Calcification and photosynthesis in reef building corals and algae. In *Ecosystems of the World. Vol. 25, Coral Reefs* (ed. Z. Dubinsky), pp. 109-131. Amsterdam: Elsevier.

Ben-Zion, M. and Dubinsky, Z. (1988). An on-line system for measuring photosynthetic characteristics via an oxygen-electrode. *J. Plankton Res.* **10**, 555-558.

Bradford, M. M. (1976). Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein dye binding. *Anal. Biochem.* **72**, 248-254.

Brown, B. E., Ambarsari, I., Warner, M. E., Fitt, W. K., Dunne, R. P., Gibb, S. W. and Cummings, D. G. (1999). Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. *Coral Reefs* **18**, 99-105.

Brown, B. E., Downs, C. A., Dunne, R. P. and Gibb, S. W. (2002a). Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. *Mar. Ecol. Prog. Ser.* **242**, 119-129.

Brown, B. E., Downs, C. A., Dunne, R. P. and Gibb, S. W. (2002b). Preliminary evidence for tissue retraction as a factor in photoprotection of corals incapable of xanthophyll cycling. *J. Exp. Mar. Biol. Ecol.* **277**, 129-144.

Butler, W. L. (1972). On the primary nature of fluorescence yield changes associated with photosynthesis. *Proc. Natl. Acad. Sci. USA* **69**, 3420-3422.

Coles, S. L. and Brown, B. E. (2003). Coral bleaching – capacity for acclimation and adaptation. *Adv. Mar. Biol.* **46**, 183-223.

De Beer, D., Kuhl, M., Stambler, N. and Vaki, L. (2000). A microsensor study of light enhanced Ca^{2+} uptake and photosynthesis in the reef-building hermatypic coral *Favia* sp. *Mar. Ecol. Prog. Ser.* **194**, 75-85.

Downs, C. A., Fauth, J. E., Halas, J. C., Dustan, P., Bemiss, J. and Woodley, C. M. (2002). Oxidative stress and seasonal coral bleaching. *Free Radical Biol. Med.* **33**, 533-543.

Dubinsky, Z. and Stambler, N. (1996). Eutrophication, marine pollution and coral reefs. *Global Change Biol.* **2**, 511-526.

Dubinsky, Z., Falkowski, P. G., Porter, J. W. and Muscatine, L. (1984). Absorption and utilization of radiant energy by light-and shade-adapted colonies of the hermatypic coral *Stylophora pistillata*. *Proc. R. Soc. Lond. B* **222**, 203-214.

Dunlap, W. C. and Chalker, B. E. (1986). Identification and quantitation of near IV absorbing compounds (S-320) in hermatypic scleractinian. *Coral Reefs* **5**, 155-159.

Dunn, S. R., Bythell, J. C., Le Tissier, M. D. A., Burnett, W. J. and Thomason, J. C. (2002). Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia* sp. *J. Exp. Mar. Biol. Ecol.* **272**, 29-53.

Dykens, J. A. and Shick, J. M. (1982). Oxygen production by endosymbiotic algae controls superoxide dismutase activity in their animal host. *Nature* **297**, 579-580.

Dykens, J. A., Shick, J. M., Benoit, C., Buettner, G. R. and Winston, G. W. (1992). Oxygen radical production in the sea-anemone *Anthopleura-elegantissima* and its endosymbiotic algae. *J. Exp. Biol.* **168**, 219-241.

Elstner, E. F. and Heupel, A. (1976). Inhibition of nitrite formation from hydroxylammonium chloride – simple assay for superoxide dismutase. *Anal. Biochem.* **70**, 616-620.

Fabricius, K. E. and Klumpp, D. W. (1995). Widespread mixotrophy in reef-inhabiting soft corals – the influence of depth, and colony expansion and contraction on photosynthesis. *Mar. Ecol. Prog. Ser.* **125**, 195-204.

Falkowski, P. G. and Owens, T. G. (1980). Light-shade adaptation: two strategies in marine phytoplankton. *Plant Physiol.* **66**, 592-595.

Falkowski, P. G. and Dubinsky, Z. (1981). Light-shade adaptation of *Stylophora pistillata,* a hermatypic coral from the Gulf of Eilat. *Nature* **289**, 172-174.

Falkowski, P. G. and Raven, J. A. (1997). *Aquatic Photosynthesis*. Massachusetts: Blackwell Science.

Falkowski, P. G., Jokiel, P. L. and Kinzie, R. A. (1990). Irradiance and corals. In *Coral Reefs. Ecosystems of the World* (ed. Z. Dubinsky), pp. 89-107. Amsterdam: Elsevier.

Fisher, T., Minnaard, J. and Dubinsky, Z. (1996). Photoacclimation in the marine alga *Nannochloropsis* sp. (Eustigmatophyte): a kinetic study. *J. Plankton Res.* **18**, 1797-1818.

Fitt, K., Brown, B. E., Warner, M. E. and Dunne, R. P. (2001). Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* **20**, 51-65.

Foote, C. S. (1976). Photosenssitized oxidation and singlet oxygen: consequences in biological systems. In *Free Radicals in Biology II* (ed. W. A. Pryor), pp. 85-133. New York: Academic Press.

Foyer, C. H., Lelandais, M. and Kunert, K. J. (1994). Photooxidative stress in plants. *Physiol. Plantarum* **92**, 696-717.

Fridovich, I. (1986). Biological effects of the superoxide radical. *Arch. Biochem. Biophys.* **247**, 1-11.

Glynn, P. W. (1993). Coral reef bleaching: ecological perspective. *Coral Reefs* **12**, 1-17.

Glynn, P. W. (1996). Coral reef bleaching: Facts, hypotheses and implications. *Global Change Biol.* **2**, 495-509.

Goericke, R. and Welschmeyer, N. A. (1992). Pigment turnover in the marine diatom *Thalassiosira weissflogii*. II. The $^{14}CO_2$-labeling kinetics of carotenoids. *J. Phycol.* **28**, 507-517.

Goiran, C., AlMoghrabi, S., Allemand, D. and Jaubert, J. (1996). Inorganic carbon uptake for photosynthesis by the symbiotic coral/dinoflagellate association. 1. Photosynthetic performances of symbionts and dependence on sea water bicarbonate. *J. Exp. Mar. Biol. Ecol.* **199**, 207-225.

Gorbunov, M. Y., Kolber, Z. S. and Falkowski, P. G. (1999). Measuring photosynthetic parameters in individual algal cells by Fast Repetition Rate fluorometry. *Photosynth. Res.* **62**, 141-153.

Gorbunov, M. Y., Falkowski, P. G. and Kolber, Z. S. (2000). Measurement of photosynthetic parameters in benthic organisms in situ using a SCUBA-based fast repetition rate fluorometer. *Limnol. Oceanog.* **45**, 242-245.

Gorbunov, M. Y., Kolber, Z. S., Lesser, M. P. and Falkowski, P. G. (2001). Photosynthesis and photoprotection in symbiotic corals. *Limnol. Oceanog.* **46**, 75-85.

Graus, R. R. and Macintyre, I. G. (1982). Variation in growth forms of the reef coral *Montastrea annularis* (Ellis and Solander): A quantitative evaluation of growth response to light distribution using computer simulation. *Smithson. Contrib. Mar. Sci.* **12**, 441-464.

Haramati, L., Achituv, Y. and Dubinsky, Z. (1997). Morphology, photoadaptation and autotrophy in hermatypic corals. *Proc. 8th Int. Coral Reef Symp. Panama* **1**, 855-860.

Hoegh-Guldberg, O. (1999). Coral bleaching, climate change and the future of the world's coral reefs. *Mar. Freshwater Res.* **50**, 839-866.

Hoegh-Guldberg, O. and Jones, R. J. (1999). Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. *Mar. Ecol. Prog. Ser.* **183**, 73-86.

Jeffrey, S. W. and Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a,b,c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* **167**, 191-194.

Jeffrey, S. W., Mantoura, R. F. C. and Wright, S. W. (1997). *Phytoplankton Pigments in Oceanography.* Paris: UNESCO Publishing.

Johannes, R. E. and Wiebe, W. J. (1970). Method for determination of coral tissue biomass and composition. *Limnol. Oceanog.* **15**, 822-824.

Jokiel, P. L. (1980). Solar ultraviolet radiation and coral reef epifauna. *Science* **207**, 1069-1071.

Jokiel, P. L. (2004). Temperature stress and coral bleaching. In *Coral Health and Disease* (ed. E. Rosenberg and Y. Loya), pp. 401-426. Berlin: Springer.

Jokiel, P. L. and Coles, S. L. (1990). Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. *Coral Reefs* **8**, 155-162.

Kolber, Z. and Falkowski, P. G. (1993). Use of active fluorescence to estimate phytoplankton photosynthesis *in situ*. *Limnol. Oceanog.* **38**, 1646-1665.

Kuhl, M., Cohen, Y., Dalsgaard, T., Jorgensen, B. B. and Revsbech, N. P. (1995). Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH, and light. *Mar. Ecol. Prog. Ser.* **117**, 159-172.

Lesser, M. P. (1996). Exposure of symbiotic dinoflagellates to elevated temperatures and ultraviolet radiation causes oxidative stress and inhibits photosynthesis in symbiotic dinoflagellates. *Limnol. Oceanog.* **41**, 271-283.

Lesser, M. P. (2000). Depth-dependent photoacclimation to solar ultraviolet radiation in the Caribbean coral *Montastraea faveolata*. *Mar. Ecol. Prog. Ser.* **192**, 137-151.

Lesser, M. P. and Shick, J. M. (1989a). Effects of irradiance and ultraviolet radiation on photoadaptation in the zooxanthellae of *Aiptasia pallida* – primary production, photoinhibition, and enzymic defenses against oxygen toxicity. *Mar. Biol.* **102**, 243-255.

Lesser, M. P. and Shick, J. M. (1989b). Photoadaptation and defenses against oxygen toxicity in zooxanthellae from natural populations of symbiotic cnidarians. *J. Exp. Mar. Biol. Ecol.* **134**, 129-141.

Lesser, M. P. and Gorbunov, M. Y. (2001). Diurnal and bathymetric changes in chlorophyll fluorescence yields of reef corals measured *in situ* with a fast repetition rate fluorometer. *Mar. Ecol. Prog. Ser.* **212**, 69-77.

Lesser, M. P. and Farrell, J. H. (2004). Exposure to solar radiation increases damage to both host tissues and algal symbionts of corals during thermal stress. *Coral Reefs* **23**, 367-377.

Lesser, M. P., Mazel, C., Phinney, D. and Yentsch, C. S. (2000). Light absorption and utilization by colonies of the congeneric hermatypic corals *Montastraea faveolata* and *Montastraea cavernosa*. *Limnol. Oceanog.* **45**, 76-86.

Levy, O., Dubinsky, Z. and Achituv, Y. (2003). Photobehavior of stony corals: responses to light spectra and intensity. *J. Exp. Biol.* **206**, 4041-4049.

Levy, O., Dubinsky, Z., Schneider, K., Achituv, Y., Zakai, D. and Gorbunov, M. Y. (2004). Diurnal hysteresis in coral photosynthesis. *Mar. Ecol. Prog. Ser.* **268**, 105-117.

Levy, O., Achituv, Y., Dubinzky, Z. and Erez, J. (2006a). Diurnal polyp expansion behavior in stony corals may prevent carbon limitation. *J. Exp. Mar. Biol. Ecol.* (in press).

Levy, O., Achituv, Y., Yacobi, Y. Z., Stambler, N. and Dubinsky, Z. (2006b). The impact of spectral composition and light periodicity on the activity of two antioxidant enzymes (SOD & CAT) in the coral *Favia favus*. *J. Exp. Mar. Biol. Ecol.* (in press).

Marra, J. (1980). Time course of light intensity adaptation in a marine diatom. *Mar. Biol. Lett.* **1**, 175-183.

Miller, K. J. (1994). Morphological variation in the coral genus *Platygyra*: environmental influences and taxonomic implications. *Mar. Ecol. Prog. Ser.* **110**, 19-28.

Muller-Parker, G. (1984). Photosynthesis-irradiance responses and photosynthetic periodicity in the sea anemone *Aiptasia pulchella* and its zoocanthellae. *Mar. Biol.* **82**, 225-232.

Muller-Parker, G., Lee, K. W. and Cook, C. B. (1996). Changes in the ultrastructure of symbiotic zooxanthellae *(Symbiodinium* sp., Dinophyceae) in fed and starved sea anemones maintained under high and low light. *J. Phycol.* **32**, 987-994.

Nii, C. M. and Muscatine, L. (1997). Oxidative stress in the symbiotic sea anemone *Aiptasia pulchella* (Carlgren, 1943): Contribution of the animal to superoxide ion production at elevated temperature. *Biol. Bull.* **192**, 444-456.

Osmond, C. B. (1994). What is photoinhibition: some insights from comparisons of sun and shade plants. In *Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field* (ed. N. R. Baker and J. R. Bowyer), pp. 95-110. Oxford: BIOS Scientific Publishers.

Owens, T. G., Falkowski, P. G. and Whitledge, T. E. (1980). Diel periodicity in cellular chlorophyll content in marine diatoms. *Mar. Biol.* **59**, 71-77.

Oyanagui, Y. (1984). Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Analyt. Biochem.* **142**, 290-296.

Porter, J. W. (1976). Autotrophy, heterotrophy, and resource partitioning in Caribbean reef building corals. *Am. Nat.* **110**, 731-742.

Porter, J. W., Muscatine, L., Dubinsky, Z. and Falkowski, P. G. (1984). Primary production and photoadaption in light and shade adapted colonies of the symbiotic coral *Stylophora pistillata*. *Proc. R. Soc. Lond. B* **22**, 161-180.

Post, A. F., Dubinsky, Z., Wyman, K. and Falkowski, P. G. (1984). Kinetics of light-intensity adaptation in a marine planktonic diatom. *Mar. Biol.* **83**, 231-238.

Rosenberg, E. and Loya, Y. E. (2004). *Coral Health and Disease*. Berlin: Springer-Verlag.

Rowan, K. S. (1989). *Photosynthetic Pigments of Algae.* Cambridge: Cambridge University Press.

Sebens, K. P., Witting, J. and Helmuth, B. (1997). Effects of water flow and branch spacing on particle capture by the reef coral *Madracis mirabilis* (Duchassaing and Michelotti). *J. Exp. Mar. Biol. Ecol.* **211**, 1-28.

Shick, J. M. and Dunlap, W. C. (2002). Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.* **64**, 223-262.

Shick, J. M., Lesser, M. P., Dunlap, W. C., Stochaj, W. R., Chalker, B. E. and Won, J. W. (1995). Depth dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellae coral *Acropora microphthalma*. *Mar. Biol.* **122**, 41-51.

Shick, J. M., Lesser, M. P. and Jokiel, P. L. (1996). Effects of ultraviolet radiation on corals and other organisms. *Global Change Biol.* **2**, 527-545.

Sokal, R. R. and Rohlf, F. J. (1981). *Biometry*, 2nd edition. New York: W. H. Freeman.

Stambler, N. and Dubinsky, Z. (2004). Stress effects on metabolism of hermatypic coral. In *Coral Health and Disease* (ed. E. Rosenberg and Y. Loya), pp. 195-215. Berlin: Springer-Verlag.

Tchernov, D., Gorbunov, M. Y., Vargas, C., Yadav, S. N., Milligan Allen. J., Häggblom, M. and Falkowski, P. G. (2004). Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proc. Natl. Acad. Sci. USA* **1001**, 13531-13535.

Todd, P. A., Sidle, R. C. and Lewin-Koh, N. J. I. (2004). An aquarium experiment for identifying the physical factors inducing morphological change in two massive scleractinian corals. *J. Exp. Mar. Biol. Ecol.* **299**, 97-113.

Vareschi, E. and Fricke, H. (1986). Light responses of a scleractinian coral (*Plerogyra sinuosa*). *Mar. Biol.* **90**, 395-402.

Walker, D. (1992). *Energy, Plants and Man*, 2nd edition. East Sussex: Oxygraphics Limited.

Warner, M. E., Fitt, W. K. and Schmidt, G. W. (1999). Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proc. Natl. Acad. Sci. USA* **96**, 8007-8012.

Yacobi, Y. Z., Pollingher, U., Gonen, Y., Gerhardt, V. and Sukenik, A. (1996). HPLC analysis of phytoplankton pigments from Lake Kinneret with special reference to the bloom-forming dinoflagellate *Peridinium gatunense* (Dinophyceae) and chlorophyll degradation products. *J. Plankton Res.* **18**, 1781-1796.

Zapata, M., Ayala, A. M., Franco, J. M. and Garrido, J. L. (1987). Separation of chlorophylls and their degradation products in marine phytoplankton by reverse-phase high-performance liquid chromatography. *Chromatographia* **23**, 26-30.