Light and Heat Stress Adaptation of the Symbionts of Temperate and Coral Reef Foraminifers Probed *in Hospite* by the Chlorophyll *a* Fluorescence Kinetics

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Since the early 80's massive bleaching affects the reef ecosystem. It involves, besides corals, several other species among which large foraminifers, and it corresponds to the loss of their photosynthetic symbionts or the symbionts' pigments. The cause is unclear, though temperature elevation and strong irradiation have been considered to be primary factors. In this work we investigated in two genera of coral reef foraminifers (*Amphistegina lobifera* and *Amphisorus heimprichii*) and in the temperate foraminifer *Sorites variabilis* the response of photosystem II (PSII) of their symbionts *in hospite* upon light stress (white light of 550 μE m^{-2} s^{-1} and red light of 3200 μE m^{-2} s^{-1}) and heat stress (up to 32 °C), by means of the Chla fluorescence transients O-J-I-P they exhibit upon illumination. The transients were analysed according to the JIP-test which leads to the calculation of several structural and functional parameters providing a quantification of PSII behaviour. We observed that the various parameters undergo modifications that differ concerning both their extent and their degree of elasticity, thus indicating that different adaptive strategies are employed in response to stress. The most pronounced of these regulatory changes is a wide decrease of the quantum yield of electron transport. However, the extent of the changes, different for the three studied species, was in general smaller when the cultures were kept under low light (70 μE m^{-2} s^{-1}) than in darkness. By the applied stressors, PSII was not damaged and, except for some cells in which an expulsion of symbionts was initiated, no bleaching was observed. This can be well correlated with the observed adaptability of PSII. As a working hypothesis, it is proposed that the decrease of the capacity for electron transport activity might be among the factors triggering bleaching in the field.

Introduction

Since the early 80's large-scale bleaching events have affected the reef ecosystem at different places of the world (Williams and Bunkley-Williams, 1990; Glynn, 1996; Pêcheux, 1999). Bleaching involves not only corals but also all other cnidarians, molluscs, sponges, ascidians and large foraminifers in symbiotic association with diatoms, dinoflagellates or cyanobacteria. Bleaching corresponds to the loss of the symbionts or their photosynthetic pigments, hence the discoloration. Subsequent mortality is highly variable. All recent bleaching events have been correlated with prolonged exposure to elevated sea-water temperatures, though it is not yet clear whether the hosts or the symbionts are more susceptible and thus responsible for the symbiosis rupture. Strong irradiation is also assumed to cause bleaching in synergism with elevated temperature. There are also indications for a similar role of UV radiation and low salinity (Coles and Jokiel, 1987) and possibly of sea-water acidification due to the rising CO_2 concentration (Pêcheux, 1999).

From studies of land plant stress it is known that photosystem II (PSII) is one of the most sensitive components of the photosynthetic apparatus towards both heat and light stress. Therefore, there were several attempts to investigate the behaviour of PSII of the photosynthetic symbionts upon bleaching-like conditions. Some of these studies used also Chl *a* fluorescence to probe PSII behaviour in corals associations or their isolated symbionts (Iglesias-Prieto *et al.*, 1992; Iglesias-Prieto, 1995; Warner *et al.*, 1996). The implication of PSII in coral reef bleaching has become a strong

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consideration, as there are evidences of both its thermolability and its sensitivity to high light intensities, attributed as photoinhibition.

In the present work we probed the PSII behaviour of the symbionts of large foraminifers *in hospite* by the Chla fluorescence transient O-J-I-P (Strasser *et al.*, 1995) they exhibit upon illumination (Tsimilli-Michael *et al.*, 1998). The PSII behaviour was quantified by a constellation of fluxes and yields, calculated by the analysis of the transients according to the JIP-test (Strasser and Strasser, 1995; Strasser *et al.*, 1999).

We studied the response of PSII to heat (up to 32 °C) and light stress (white light of 550 μE m^{-2} s^{-1} and red light of 3200 μE m^{-2} s^{-1}) in three genera of large foraminifers: *Amphistegina lobifera*, harbouring as symbiont the diatom *Fragilaria* sp., and *Amphisorus heimprichii* and *Sorites variabilis* who carry the same symbiont as corals (*Symbiodinium* sp.). *Amphistegina* and *Amphisorus* are main components of the coral reef ecosystem producing a large amount of $CaCO_3$, while *Sorites* is a temperate foraminifer. Thus, we could also compare the behaviour of PSII of different symbionts from the same natural habitat, and of the same symbiont from different habitats.

The aim was to analyse the response of the symbionts upon light and heat stress in order to investigate whether the stresses were destructive, or whether and how the symbionts would manage to undergo adaptive processes revealed as regulations of the different parameters quantifying the PSII behaviour. The final goal was to correlate adaptability with resistance to bleaching, which might further help to get an access to the understanding of the causes of bleaching.

Material and Methods

Amphistegina and *Amphisorus* were collected in Mauritius and *Sorites* in the Mediterranean sea near Nice, at 1.5 meters depth. For the experimentation, the foraminifers were selected, cleaned and distributed in glass-tubes with 5.5 ml of daily exchanged Mediterranean sea-water (pCO_2-controlled pH at about 8.2), kept in a thermostated water bath with a temperature gradually elevated from 25 to 32 °C, and exposed to light-dark cycles: 12 h light at 70 μE m^{-2} s^{-1} (L) - 12 h dark (D), as shown in Fig. 1. The fluorescence measurements were conducted every 6 hours. Between the 19th and 20th measuring periods, the light of 70 μE m^{-2} $1nbs^{-1}$ was substituted by a light of 550 μE m^{-2} s^{-1} (high light, HL). Two parallel sets of tubes were used, each of them measured with a different mode. For the one set the fluorescence transients were recorded for 5 s, preceded by 1 min dark (mode A). The physiological state of the photosynthetic apparatus when measured with mode A is a dark adapted or low-light adapted state and has thus been denoted as State-1 (S_1) (Tsimilli-Michael *et al.*, 1998). The other set was measured as shown in Fig. 1 (mode B): The 2 min – illumination leads to a physiological state denoted as State-2 (S_2) (Tsimilli-Michael *et al.*, 1998). The fluorescence decrease upon the S_1 to S_2 transition is reversible except for a small residual strain. Therefore, we have made the distinction between the State-1 recorded under mode B, which carries the "memory" of the previous S_1 to S_2 transitions (denoting it as S_{11} state), and the S_1 state (recorded under mode A) which has not a history of such transitions (for more details see Tsimilli-Michael *et al.*, 1998).

The Chl *a* fluorescence transients of the foraminifer symbionts *in hospite* were measured by a PEA fluorimeter (Plant Efficiency Analyser, built by Hansatech Instruments Ltd. King's Lynn Norfolk, PE 30 4NE, GB) and recorded up to 5 s or 2 min with a 12-bit resolution (Strasser *et al.*, 1995). The data acquisition is: every 10 μs for the first 2 ms, every 1 ms between 2 ms and 1 s, and every 100 ms thereafter. The fluorescence transients were induced by a red light (peak at 650 nm) of 600 W m^{-2} (3200 μE m^{-2} s^{-1}) provided by an array of six LEDs.

Each transient was analysed according to the JIP-test (Strasser and Strasser, 1995; Strasser *et al.*, 1999), which utilises the following original data: the maximal measured fluorescence intensity, F_P, equal here to F_M since the excitation intensity is high enough to ensure the closure of all reaction centres (RCs) of PSII; the fluorescence intensity at 50 μs considered as the intensity F_0 when all RCs are open; the fluorescence intensities at 150 μs, at 2 ms (J step) denoted as F_J, and at 60 ms (I-step) denoted as F_I. For the quantification of PSII behaviour we used the set of formulae shown in Table I, by which the following biophysical parameters, all referring to time zero (onset of flu-

Table I. The formulae of the JIP-test used in the present work.

Specific fluxes	Yields	Yields as ratios of fluxes
$TR_0/RC = (M_0/V_J)$	$\varphi_{Po} = [1 - (F_0/F_M)]$	$\varphi_{Po} = (TR_0/RC)/(ABS/RC)$
$ET_0/RC = (M_0/V_J) * (1\text{-}V_J)$	$\varphi_{E0} = [1 - (F_0/F_M)] * (1\text{-}V_J)$	$\varphi_{E0} = (ET_0/RC)/(ABS/RC)$
$ABS/RC = (M_0/V_J) / [1 - (F_0/F_M)]$	$\psi_0 = (1\text{-}V_J)$	$\psi_0 = (ET_0/RC)/(TR_0/RC)$

where, $V_J = (F_J - F_0) / (F_M - F_0)$ and $M_0 = 10 * (F_{150\,\mu s} - F_0) / (F_M - F_0)$

orescence induction), were calculated: (a) the specific energy fluxes (per reaction centre) for absorption (ABS/RC), trapping (TR_0/RC) and electron transport (ET_0/RC) and, (b) the flux ratios or yields, i.e. the maximum quantum yield of primary photochemistry (φ_{Po} =TR_0/ABS), the efficiency (ψ_0= ET_0/TR_0) with which a trapped exciton can move an electron into the electron transport chain further than Q_A^-, and the quantum yield of electron transport ($\varphi_{Eo} = \varphi_{Po} * \psi_0$= ET_0/ABS).

Results

The O-J-I-P fluorescence transient of the studied species, here denoted as O-J-I-G-H due to the appearance of more steps (Tsimilli-Michael *et al.*, 1998), depends strongly on the light and temperature cultivation conditions, as shown for example in Fig. 2. As no changes of the F_0 level were observed, except for a slight and reversible decrease after the 6 hours in HL (data not shown) or upon the S_{11} to S_2 transition (Fig. 1), we express the fluorescence values by the F_t/F_0 ratios, thus avoiding fluctuations due to the movement of the cells in the glass tubes.

The shape of the transient depends on the cultivation light conditions (light-dark) while the amplitude of variable fluorescence decreases upon the temperature elevation. However, the decrease of F_t/F_0 occurs mainly when the cultures were in the dark phase, while in the light phase (70 $\mu E\ m^{-2}\ s^{-1}$) only minor decreases take place (Fig. 2). This is an expression of a low-light thermoprotection (Havaux and Strasser, 1990; Srivastava and Strasser, 1996), here realised by reversing the deformations occurring in the dark. The transients do not show the K-step (at 300 µs) which appears when a reduction of the oxygen evolving capacity occurs

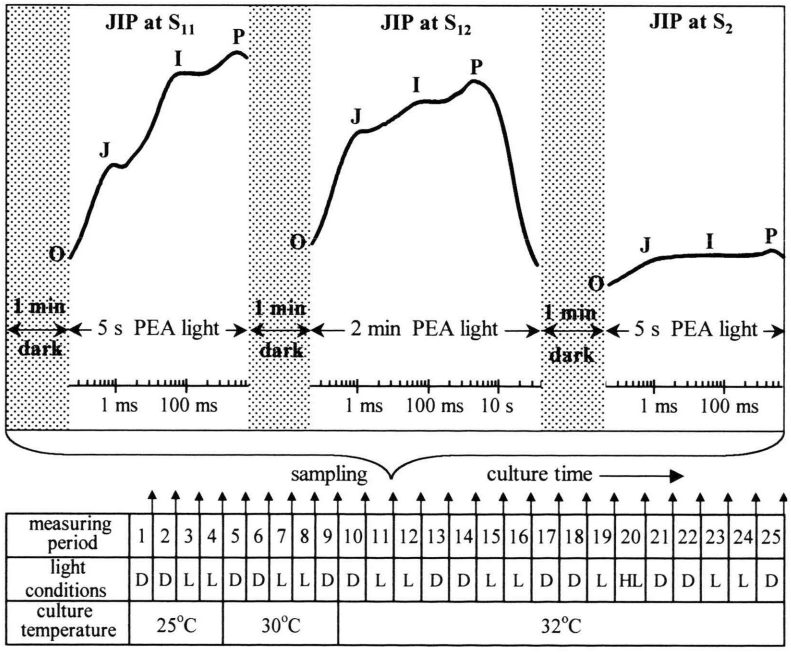

Fig. 1. The experimental protocol showing the light conditions and the temperature of the culture for the 6 h preceding each measuring period. An example of three successive fluorescence transients at S_{11}, S_{12} and S_2 states, recorded according to mode B at the 11th measuring period, is also included. The transients are presented on a logarithmic time scale. For other details see text.

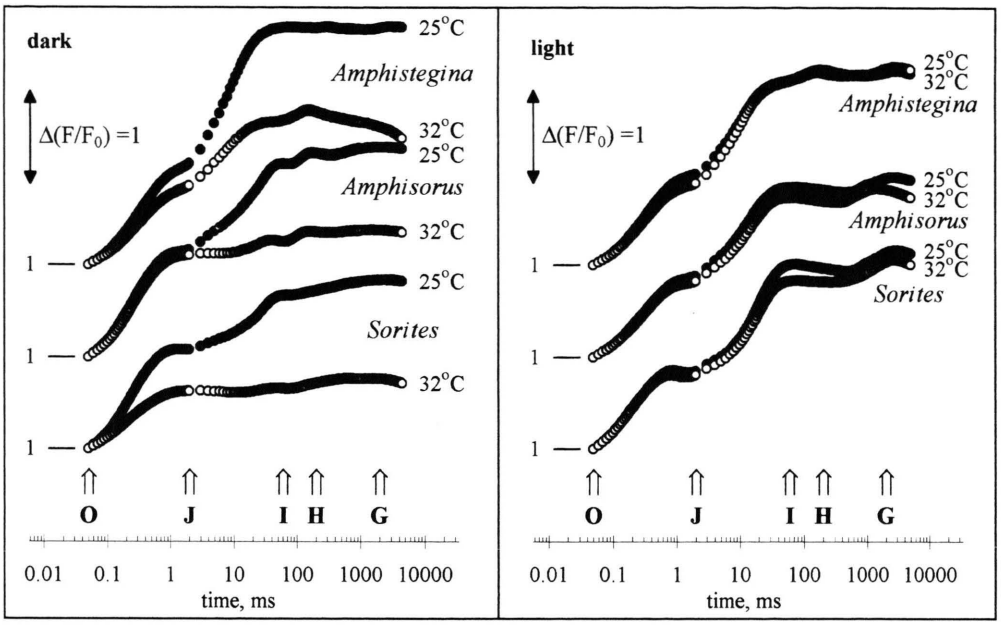

Fig. 2. The fast fluorescence transients of the three studied species being for 12 hours in the dark or in the light of 70 μE m^{-2} s^{-1}, at two cultivation temperatures, 25 °C (2nd and 4th measuring period respectively) and 32 °C (14th and 16th measuring period respectively). The transients, vertically displaced for clarity, are presented on a logarithmic time scale, revealing a sequence of more steps after the J-step, i.e. O-J-I-H-G. Depending on the experimental conditions any step can be the highest (P-step).

(Srivastava *et al.*, 1997; Strasser, 1997; Tsimilli-Michael *et al.*, 1998).

The patterns of the ratios F_M/F_0, F_I/F_0 and F_J/F_0 during the whole experiment are shown in Fig. 3 for the S_{11} state. It is worth pointing out that at the first measuring period in the dark at 25 °C the F_M/F_0 and F_I/F_0 show higher values than in the following light phase, as happens normally in higher plants where the fluorescence values are lower at any light-adapted state that at the dark-adapted state. Upon the temperature elevation, *Sorites* show the most and *Amphistegina* the less wide decreases of the F_t/F_0 ratios, both in the dark and in the light, indicating that the different species acquire different physiological sensitivities to heat. The difference between the two soritids (*Amphisorus* and *Sorites*) hosting also the same symbiont might be due to the long-term adaptation in their different natural habitats. Upon a further exposure to 32 °C the values in the dark appear slightly restored (except in *Sorites*) possibly indicating some hardening of the system. The 6 h exposure to high light (HL, 550 μE m^{-2} s^{-1}) results in a decrease of the F_t/F_0 ratios, which eventually recovers.

With the ratios presented in Fig. 3 the fluorescence transients can be described and compared, but only at the phenomenological level. In order to deduce biophysical information and get an insight into the PSII behaviour, we analysed the transients according to the JIP-test (see Materials and Methods). The results to be further presented will refer to the case of *Amphisorus* since the other two species show similar patterns, however with a sensitivity difference in the same order as manifested in Fig. 3.

From the patterns of Fig. 4 it is demonstrated that the various specific fluxes and yields undergo deformations of different extent, thus indicating that different survival strategies are employed in response to stress. Moreover, they show differences concerning the extent of low-light thermoprotection. ψ_o appears more sensitive to the cultivation conditions than φ_{Po}. Though they are completely independent, their changes are in phase and, thus, the changes of φ_{Eo} are even more

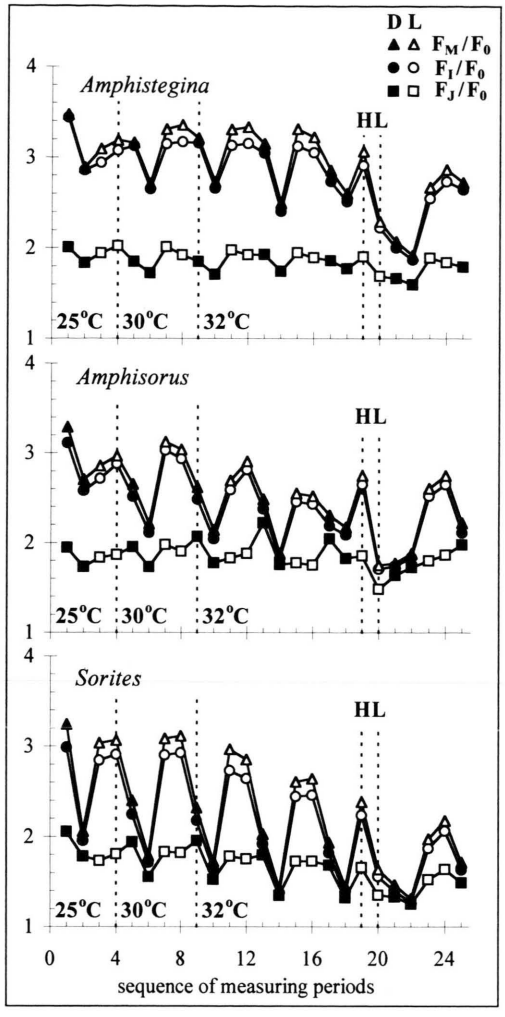

Fig. 3. The response of the three species to the sequence of light-dark cycles, under a gradually elevated cultivation temperature, and for a temporary (6 hours) increase of the light intensity to 550 μE m^{-2} s^{-1} (HL, high light), expressed by the fluorescence ratios F_M/F_0, F_I/F_0 and F_J/F_0. The results refer to the S_{11} state. Closed symbols stand for the dark phase (D) and open symbols for the light phase (L) of 70 μE m^{-2} s^{-1}.

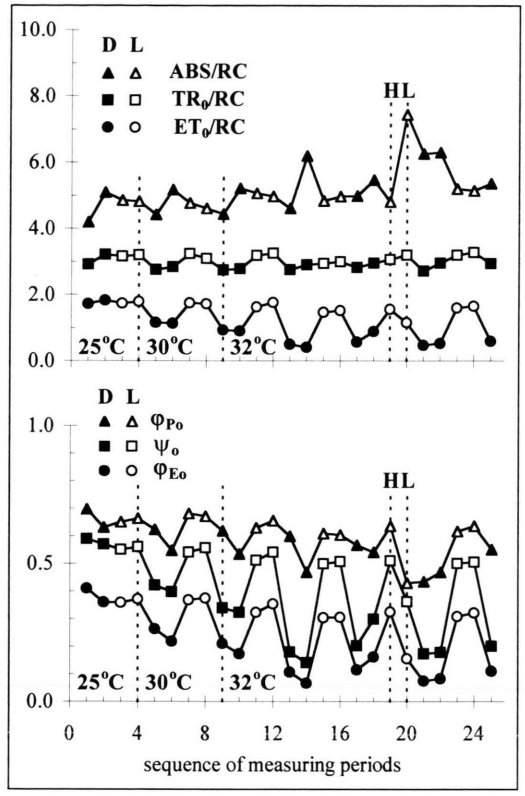

Fig. 4. The PSII behaviour in *Amphisorus* described by the patterns of the specific fluxes ABS/RC, TR_0/RC, ET_0/RC (upper plot) and the yields or ratios of fluxes (lower plot). For other details see legend φ_{Po}, ψ_o and φ_{Eo} of Fig. 3.

pronounced ($\varphi_{Eo} = \varphi_{Po} * \psi_0$). Accordingly, the specific flux $ET_0/RC = TR_0/RC * \psi_0$ is more sensitive than TR_0/RC.

It is worth to focus here on the comparison of the three parameters probing primary photochemistry, reminding also their relation: φ_{Po} = (TR_0/RC)/(ABS/RC). The quantum yield φ_{Po} refers to the whole sample averaging the yield of all photosynthetic units. An increase of ABS/RC, which gives a measure of the average antenna size, i.e. of the total chlorophyll molecules excited per active RC, indicates the inactivation of a fraction of RCs which, due to the stability of F_0, we can attribute to their transformation to quenching sinks (Krause *et al.*, 1990; Krüger *et al.*, 1997; Tsimilli-Michael *et al.*, 1998). On the other hand, TR_0/RC is an expression of the photochemical activity of the active RCs. From Fig. 4 we see that the decrease of φ_{Po} upon the temperature elevation is related to the increase of ABS/RC, while its oscillations are due to the oscillations of TR_0/RC which are independent of the temperature regarding both their amplitude and base line. The increase of ABS/RC is also responsible for the decrease of φ_{Po} caused by the exposure to HL, while TR_0/RC does not appear to sense it.

A similar analysis of the transients at the S_2 state showed that all the yields, as well as ET_0/RC, are much lower than at S_{11} (see e.g. the φ_{P0} patterns in Fig. 5), while TR_0/RC is the same and ABS/RC is much higher. Therefore, the decrease of φ_{P0} upon the S_{11} to S_2 transition appears to result from the inactivation of a big fraction of RCs, while the photochemical activity of the still active is not affected. As shown in Fig. 5, the fractional changes of φ_{Po} upon L-D are almost the same in S_2 and S_{11}.

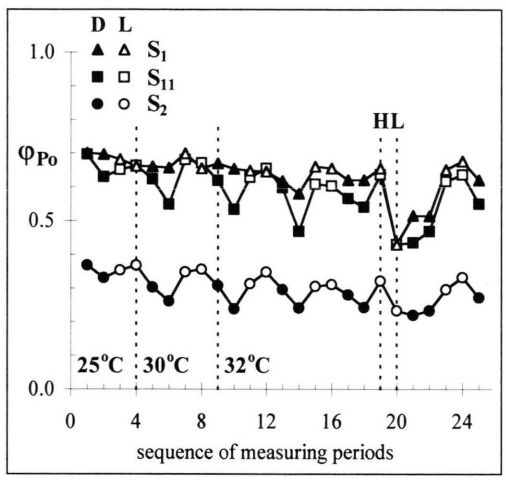

Fig. 5. The pattern of φ_{Po} in *Amphisorus* being at S_1, S_{11} and S_2. For other details see legend of Fig. 3.

In Fig. 5 we also present for comparison the pattern of φ_{Po} for the S_1 state. The deviation of the S_{11} from the S_1 values expresses the residual strain of φ_{Po} after the recovery from the S_{11} to S_2 transition which results, as calculated, from an incomplete reactivation of the RCs that were inactivated at S_2. As shown in Fig. 5, the residual strain is smaller when the cultures are in the light phase, which indicates that low light enhances the reactivation of the RCs. On the other hand, the deformations of ψ_0 and, concomitantly, of ET_0/RC were found to be highly elastic. Therefore, the plasticity of φ_{Eo} is mainly due to the plasticity of φ_{Po}. (For the definition of plasticity, elasticity and residual strain, see Krüger *et al.*, 1997).

The response of the photosynthetic apparatus of the symbionts to the 2 min – illumination by the red light of 3200 μE m^{-2} s^{-1} shows that the applied heat stress did not affect its capability to undergo regulatory changes. Furthermore, it can be speculated that, even though the light used is an artificial light and S_2 as such can not be induced in the field, the modifications occurring upon the S_{11} – S_2 transition show the trend of the effect of strong light in the natural habitat of foraminifers, and the behaviour at S_{11} shows the trend of the recovery that follows. Similar trends are also observed after the exposure to HL. It is worth to mention that, during the diurnal changes in the tropics at summer time, the photosynthetic photon flux density (PPFD) at the water surface is above 1500 μE m^{-2} s^{-1} for about 6 hours (Merino *et al.*, 1995) and can be as high as 2000 μE m^{-2} s^{-1}. Moreover, the PPFD recorded in the water shows brief flashes with 60% higher PPFD than that measured at the water surface, due to light focusing and defocusing by the waves (Merino *et al.*, 1995). Taking into account the light absorption by a water layer of 1.5 m, we can estimate that the foraminifers receive *in situ* a basic PPFD up to 1600 μE m^{-2} s^{-1}, which can temporarily reach the 2500 μE m^{-2} s^{-1}. We can therefore assume that the S_{11} state in our experiments is more representative of the symbionts' behaviour in the field than the S_1 state.

The behaviour of φ_{Eo}, both for the dark and the light phases of the cultivation, during the temperature elevation and for all states, S_1, S_{11}, S_{12} and S_2, is summarised in Fig. 6 for the case of *Amphisorus*. The values are the averages of all measurements at the same state and condition.

Discussion

Coral reef bleaching in the field has been correlated to elevated sea-water temperatures in the range of 28 to 34 °C. Even short-term exposure to temperatures above 30 °C is considered to trigger bleaching while a long exposure to 27–30 °C is assumed to have the same impact (Iglesias-Prieto *et al.*, 1992). For the temperate foraminifer *Sorites* the thermotolerance limits are expected to be lower, due to the lower temperature range in the Mediterranean.

Studies of the temperature effect on the photosynthetic apparatus of the symbionts under laboratory conditions are mainly dealing with the demonstration of damages of PSII and relate them to the initiation of bleaching. Such a possible damage is the denaturation of PSII, well-known from heat

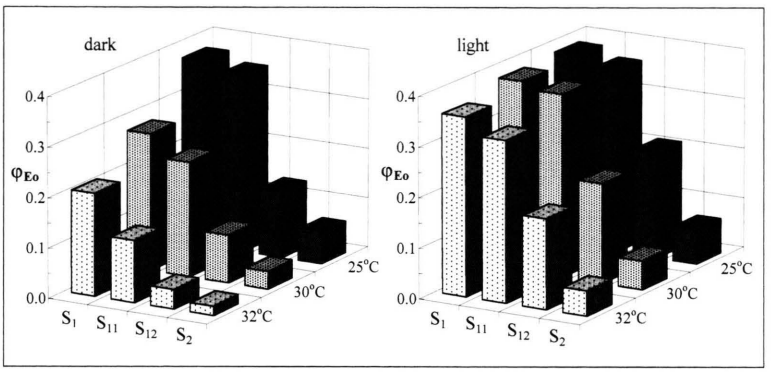

Fig. 6. The quantum yield of electron transport φ_{Eo} in *Amphisorus* at the S_1, S_{11}, S_{12} and S_2 states, vs the cultivation temperature, shown for the dark (left) and the light (70 μE m^{-2} s^{-1}) cultivation phase (right).

stress studies in land plants, which is revealed by a sharp increase of F_0 after a threshold temperature (Schreiber and Berry, 1977; Havaux, 1993). As reported by Iglesias-Prieto *et al.* (1995), this was indeed observed in isolated symbionts, but at a temperature higher than 34 °C. On the other hand, bleaching, either as loss of symbionts or of their pigments, would result in lower F_0 values. Since throughout the full course of our experiments the F_0 level remained unaffected by the temperature elevation, we can safely conclude that, in the certain temperature range used, no such damage of PSII occurred. The only change of F_0 was a slight and reversible decrease after the exposure to high light (HL, 550 μE m^{-2} s^{-1}) for 6 hours, as well as upon the strong light of the PEA-instrument (3200 μE m^{-2} s^{-1}) inducing the S_{11} to S_2 transition. Such a decrease of F_0 is often observed in higher plants when the photosynthetic apparatus is driven from the dark-adapted to a light-adapted state.

The reduction of the oxygen evolving capacity has also been reported as another possible damage. In such a case, the O-J-I-P transient would show an additional step at 300 μs, the K-step, as recently reported (Srivastava *et al.*, 1997; Strasser, 1997). This step also appears in foraminifers exposed to higher temperatures (Tsimilli-Michael *et al.*, 1998), but not in the range 25–32 °C used for the present work.

The information deduced from the fluorescence measurements are in accordance with the fact that the cells, after the end of the experiment, were not bleached. However, some *Amphistegina* and *Sorites* cells showed darker last chambers which means that some symbiont expulsion was initiated. Nevertheless, when the cells were transferred back to 25 °C they remained unbleached and fully alive for a long time.

It is generally accepted that the heat tolerance of the organisms is different under laboratory conditions than in the field where they are exposed to more stressors which may act in synergism, as well as in antagonism. High levels of natural light were found to provoke coral bleaching in synergism with elevated temperatures (Coles and Jokiel, 1978). However, in our experiments, neither the exposure for 6 h to the high light of 550 μE m^{-2} s^{-1} (HL) combined with 32 °C, nor the illumination for 2 min every 6 h by the strong light of the PEA-instrument (3200 μE m^{-2} s^{-1}) used to induce the S_{11} to S_2 transition, resulted in any damage of PSII. There are indications that another possible co-stressor in nature is low salinity and sea-water acidification (due to CO_2 rise), the synergism of which we intend to investigate in future experiments.

Let us now focus on the changes of the PSII behaviour. Already at the phenomenological level, the selected fluorescence transients in Fig. 2 and the pattern of the ratios F_M/F_0, F_I/F_0 and F_J/F_0 for the full course of the experiment (Fig. 3) show clearly that temperature elevation provokes their down regulation when the cultures are in the dark phase, while minor changes are observed in the light phase (L, 70 μE m^{-2} s^{-1}). This can be regarded as an expression of the well-known low-light thermoprotection (Havaux and Strasser, 1990; Srivastava and Strasser, 1996). However, we have to emphasise that we are not dealing here with a protection against damage, but with a partial reversion of the decrease of the F_t/F_0 values which occurs in the dark phase upon the temperature elevation.

With the JIP-test we can utilise the fluorescence transients to get an access to several parameters probing the PSII behaviour, beside the commonly used φ_{Po}. These parameters are the specific fluxes (per RC) for absorption (ABS/RC), trapping (TR_0/RC) and electron transport (ET_0/RC), and the flux ratios ψ_0 and φ_{Eo}. In our experiments φ_{Eo} appears to be the most sensitive parameter. We remind that ψ_0 is independent of φ_{Po} and that $\varphi_{Eo} = \varphi_{Po} * \psi_0 = [(RC/ABS) * (TR_0/RC)] * \psi_0$. This means that φ_{Eo} can be regulated at different levels: (a) by the transformation of RCs to quenching sinks, (b) by changes of the trapping flux per active reaction centre (TR_0/RC) caused by structural changes of the active PSII units affecting their photo-chemical and/or nonphoto-chemical de-excitation rate constants, (c) by changes of ψ_0 related to structural changes affecting the overall rate constant of the electron transport beyond Q_A^-.

Our results show that the regulation of φ_{Eo} is realised here at all three levels: The oscillations upon the alternating light-dark cycles in the pattern of φ_{Po} result from oscillations of TR_0/RC. On the other hand, the decrease of φ_{Po} upon the temperature elevation results from the inactivation of RC/ABS (increase of ABS/RC, Fig. 4). The third level of regulation refers to ψ_0 which decreases even more than φ_{Po} upon the temperature elevation.

The exposure to high light (HL) does not affect TR_0/RC, but results in an inactivation of a fraction of RCs leading to a decrease of φ_{Po}. As it also induces a decrease of ψ_0, the decrease of φ_{Eo} is much more pronounced than the decrease of φ_{Po} and ψ_0. A similar behaviour is observed upon the strong light of the PEA-instrument inducing the S_{11} to S_2 transition, but with more pronounced decreases of the affected parameters.

According to the stress concept proposed by Strasser (1988), any environmental change can be regarded as a stressful event for an organism in the sense that it disturbs the optimality it had achieved through adaptation to its environment, and leads the system to suboptimality. No kind of stress is harmful if the system manages to adapt, but any kind of stress can be destructive if it exceeds the limits of the system's adaptability. On the other hand, the adaptability of a system is defined by its capability to undergo deformations upon environmental changes, in order to achieve a new optimal situation, in harmony with the new environment. Therefore, these deformations can be regarded as manifesting the mobilisation of adaptation mechanisms, which protect the organisms (see also Tsimilli-Michael *et al.*, 1996).

In our experiments we applied a heat stress by elevating the temperature up to 32 °C and we observed, as above discussed, that the response of the symbionts was the deformation of different structural parameters, which means that different adaptive strategies were employed. We also used for each temperature a light stressor, the strong light of the PEA-instrument, to induce a state transition and we saw that the temperature elevation did not affect the capability of the photosynthetic apparatus to undergo structural deformations. These deformations were highly reversible, with a partial plasticity observed mainly in the dark, and especially after 12 h in the dark; however, the residual strain (the non-reversed part of a deformation) is only slightly increasing with temperature. Moreover, at the highest used temperature, 32 °C, we observed that PSII retained its capability to respond to the exposure for 6 h to the high light of 550 $\mu E\ m^{-2}s^{-1}$ (HL) as revealed by the almost elastic deformations of the several parameters.

In the frame of our stress concept we can therefore conclude that in our experiments the symbionts were exposed to a heat stress within the limits of their adaptability, which is in accordance with the fact that they did not show any damage. However, their adaptation was realised by structural changes which resulted in a down regulation of their photosynthetic capacity expressed by a pronounced decrease of the quantum yield for electron transport φ_{Eo}.-

From the values of the quantum yield of electron transport (shown in Fig. 6) we can draw for each temperature two curves, schematically presented by straight lines in the plot $\varphi_{Eo} = f(I_{adapt})$ of Fig. 7: the upper curve corresponds to conditions of optimal thermoprotection (values from the light cultivation phase, open symbols), and the lower curve to conditions of no thermoprotection (dark cultivation phase, closed symbols). In the natural environment of foraminifers, the pattern of the light intensity is the resultant of diurnal and spatial light changes (Merino *et al.*, 1995), as men-

tioned in the Results section. Upon such changes it is reasonable to assume that the φ_{Eo} values for each temperature are "moving" in the according zone defined between an upper and a lower curve, similar to those of Fig. 7. Since the temperature elevation shifts down these zones, the overall photosynthetic activity of the symbionts is expected to decrease with the temperature rise.

It can be reasonably assumed, in agreement with a hypothesis proposed by Iglesias-Prieto *et al.*

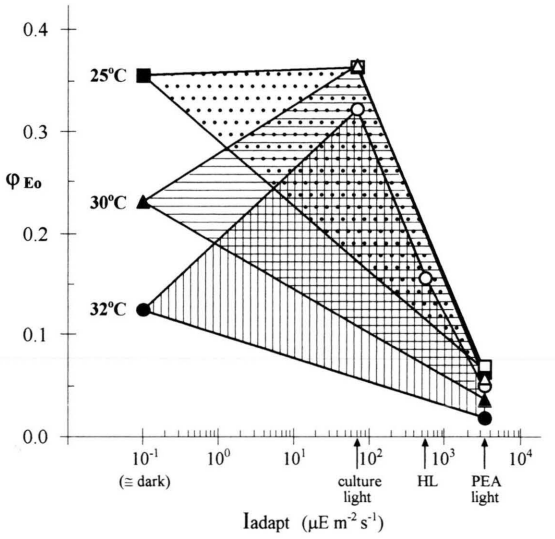

Fig. 7. A simulation of the zones where the φ_{Eo} values are expected to move upon light changes in the natural environment of foraminifers. For each temperature, the zone is defined between the upper curve corresponding to conditions of optimal thermoprotection (open symbols) and the lower curve corresponding to conditions of no thermoprotection (closed symbols). The plot is based on the results from *Amphisorus*. The light intensity is presented on a logarithmic scale. For other details see text.

(1995) that a reduction in the productivity of photosynthetic metabolites may cause a reduction of their delivery to the host. Since the symbionts' photosynthetic products are the major energy source for the symbiotic association, we speculate that, though for the symbionts as such the reduction of the productivity of metabolites is not harmful but represents an adaptive and protective procedure, it may have a negative effect on the cohabitation. It may thus trigger bleaching initiating the symbiosis rupture and the expulsion of symbionts, as we have observed in some cells in our experiments. This working hypothesis, which is supported by evidences (Iglesias-Prieto *et al.*, 1992; Warner *et al.*, 1996) that the reduction in photosynthesis precedes any significant reduction of the symbionts density while pigment loss occurs after physiological damage of photosynthesis, has to be tested both in the laboratory and in the field.

With the work presented here, it is shown that the JIP-test can be considered as a convenient and powerful non-invasive tool for studying *in vivo* the vitality and adaptability of foraminifers. Other coral reef species have been as well studied before (Tsimilli-Michael *et al.*, 1998). As we have successfully conducted fluorescence measurements *in situ* with the PEA-fluorimeter (unpublished results), we propose that the JIP-test could be used to screen the PSII behaviour in whole reef biotopes under stress caused by natural light and temperature changes, in an effort to get an access to the causes of bleaching.

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