

Perpetual adaptation in a perpetually changing environment as a survival strategy of plants: a case study in foraminifers concerning coral reef bleaching

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Abstract

Coral reef bleaching is a global phenomenon poorly understood today. We investigated during 7 d the photosynthetic behaviour of symbionts of coral reef and temperate foraminifers *in hospite*, by means of the JIP-test. By this screening test the fast fluorescence rise O-J-I-P, measured by a Plant Efficiency Analyser (PEA) with 10 μ s time resolution and 12 bit signal resolution, was analysed. It informs about the structure and function of photosystem 2 being at different physiological states established by adaptation to different irradiance and temperature. The test needs a measuring time *in vivo* of only 1 to 5 s, and thus many samples can be analysed. The measurements can be done continuously even on a single cell in a test tube or on the reef. The reef foraminifers tested here were *Amphistegina* and *Amphisorus*, freshly collected in Mauritius. As a temperate foraminifer, *Sorites* from the Mediterranean Sea was tested. The cells are very sensitive to slight temperature changes (25 to 32 °C). The comparison showed that the more the foraminifers live in an environment with constant temperature the less they are able to respond to temperature changes and, thus, the less they can adapt. Rising the temperature increases in general the sensitivity to different stress factors, such as high irradiance, pH, CO₂, etc. After the test series, the cells recovered fully and were kept in an aquarium for long time observation.

Additional key words: *Amphisorus*; *Amphistegina*; chlorophyll fluorescence kinetics; electron transport; irradiance; photosynthesis; photosystem 2; quantum yield; *Sorites*; temperature.

Introduction

Since the early 80's, mass "bleaching" affects the founder of the reef ecosystem

Received 21 January 1999, accepted 14 April 1999.

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Acknowledgements: We are thankful to Nathalie von Arnim, professor of biology, for supplying large foraminifers from Mauritius. R.J. Strasser acknowledges financial support from the Swiss National Foundation (SNF 31-46.860.96 and SNF 31-52.541.97) and M. Tsimilli-Michael from the Cyprus Ministry of Education and Culture (sabbatical leave).

(Williams and Bunkley-Williams 1990, Glynn 1993, Pêcheux 1999). It involves not only corals but also all other cnidarians, as well as molluscs, sponges, and large foraminifers, in symbiotic association with either dinoflagellates, diatoms, or cyanobacteria. Bleaching corresponds to the loss of the symbionts and/or the pigments, hence the discolouration. Subsequent mortality is highly variable. It occurs world-wide, in all reef biotopes, increasing in magnitude and becoming chronic. Spectacular shell abnormalities, almost unknown in recent and geological time, are associated with bleaching and observable in all large foraminiferous genera (Pêcheux 1999).

The cause is unclear, although certainly global. As bleaching happens preferentially in summer with a clear sky and calm sea, temperature and irradiation are certainly primary factors. But evidence of a long term warming is controversial and good counter examples are known. Ultraviolet rays have often been invoked but we found (M.P.) no change of the ozone layer in tropics, nor correlation with bleaching events. Other global factors can be weather pattern changes and general seawater acidification (21 % H^+ more) due to CO_2 rise. Whatever the figure is, bleaching demonstrates that the reef ecosystem is near its limits and that small changes of environment induce fundamental perturbation of tropical photosynthetic symbioses.

Chlorophyll (Chl) fluorescence measurements, widely used in studies of land plants stress, were recently introduced for corals or their isolated symbionts (Iglesias-Prieto 1995, Warner *et al.* 1996). The state of the photosynthetic apparatus is strongly sensitive to bleaching-like conditions and is suggested to be at the origin of the symbiosis rupture. We here apply the JIP-test (Strasser and Strasser 1995), a fast screening test for the analysis of the fast fluorescence transients (Krüger *et al.* 1997, Strasser *et al.* 1997, 1999) in order to investigate long-term temperature and irradiance effects on the photosynthetic behaviour of the symbionts of large foraminifers *in hospite*. We studied three species, particularly easy to laboratory manipulation. Two of them, *Amphistegina* and *Amphisorus*, are main components of the reef ecosystem, depositing large amounts of $CaCO_3$. At least the genus *Amphistegina* is bleaching world-wide (Hallock *et al.* 1995) and appears even more sensitive to this phenomenon than corals themselves. The third was *Sorites*, a temperate foraminifer.

Materials and methods

Foraminifera: Three symbiotic large foraminifers have been used: *Amphistegina lobifera* Larsen (1976), which harbours diatoms (*Fragilaria* sp.) as symbiont, *Amphisorus heimprichii* Ehrenberg (1839) and *Sorites variabilis* Lacroix (1941) who carry the same symbiont as corals (*Symbiodinium* sp.). *Amphistegina* and *Amphisorus* have been collected in Mauritius in back reef, 1.5 m depth, and safely transported within one day to the laboratory in Geneva. *Sorites* have been collected in the Mediterranean sea near Nice, France, also at 1.5 m depth, and cultivated during nine months in a closed seawater aquarium at 25 °C under 50-100 $\mu\text{mol}(\text{quantum})\text{ m}^{-2}\text{ s}^{-1}$

and at pH of 8.2-8.5. The maximum temperature at the Mauritius site is 30-32 °C and at the Mediterranean site 29-30 °C.

For the experiments the foraminifers were selected, cleaned, and distributed in glass-tubes of 5.5 cm³ Mediterranean seawater. 10 *Amphistegina* and 4 *Amphisorus* specimens were used per tube, but only a single cell of *Sorites*. Twelve parallel tubes for each species were kept in a thermostated water bath. Light-dark cycles were given, with 12 h irradiation (70 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and 12 h dark. The seawater was exchanged daily with pCO₂-controlled pH at either 8.00 or 8.33 (with coherent daily shift of 0.10 ± 0.05 , 40 S, standard alkalinity 2720 mM eq.). Other foraminifers as well as corals were analysed (results not shown). Their behaviour did not principally differ from the behaviour of the organisms reported here.

The JIP-test - measurement and analysis of the fast fluorescence rise: Since the discovery of the Chl *a* fluorescence transient, fluorescence measurements have become a non-invasive tool to analyse photosynthetic systems. Here we describe a method which is based on the measurement of the fast fluorescence transient by the portable instrument Plant Efficiency Analyser (PEA, *Hansatech Instruments*, King's Lynn, GB), recorded up to 5 s or 2 min with a 12-bit resolution and a data acquisition of 10 μs for the first 2 ms, 1 ms between 2 ms and 1 s, and 100 ms thereafter (for details see Strasser *et al.* 1995). The fluorescence is excited by 6 LEDs providing a red irradiance of 600 W m⁻² (630 nm). Any sample of about 4 mm² (as a tissue or in suspension or even one single foraminifer cell in a test tube or on the reef) can be measured. The analysis of the fast fluorescence rise according to the JIP-test (Strasser and Strasser 1995) allows to derive several expressions leading to the dynamic description of a photosynthetic sample at a given physiological state (Krüger *et al.* 1997, Strasser *et al.* 1997, 1999). With the JIP-test the following goals are achieved: (a) several hundred samples can be measured per hour, (b) the values are presented in a standardised form, (c) a manual is provided so that even non-specialists can measure in the field.

All oxygenic photosynthetic material investigated so far show a polyphasic fluorescence rise during the first second of irradiation, *i.e.*, phases O, J, I, P (Strasser and Govindjee 1992, Strasser *et al.* 1995). This OJIP-transient changes its shape according to many environmental conditions, such as irradiance (Tsimilli-Michael *et al.* 1995, 1996, Krüger *et al.* 1997, Srivastava and Strasser 1997), temperature (Srivastava *et al.* 1997, Strasser B. 1997), drought (Van Rensburg *et al.* 1996), or chemical influences (Ouzounidou *et al.* 1997). From the original values stored during the first 5 s, the following values are retained and used to express ratios and biophysical expressions: the maximal measured fluorescence intensity, F_M or F_P ; the fluorescence intensity at 50 μs (F_0), 150 μs , 300 μs , 2 ms (denoted as F_J), and at 60 ms (where the I-step appears in foraminifers, hence denoted as F_I). From the fluorescence values at 50 and 150 μs , the initial slope M_0 is calculated (see Table 1). The JIP-test refers to the main steps J, I, and P. However, a full transient showed here a sequence of more than one step between the step J and the fluorescence maximum. Therefore, the polyphasic transient is hereafter denoted as O-K-J-I-H-G, where the labelling follows an alphabetic order from the slower to the faster phases. The very

fast (300 μ s) K-step appears and becomes dominant if the cells suffer under strong heat (Srivastava *et al.* 1997, Strasser B. 1997). Each step can be the highest and would then be denoted as F_M or P.

The model used for the photosynthetic system: A highly simplified working model of the energy fluxes in photosystem 2 (PS2) (Fig. 1) has been reported by Strasser and Strasser (1995) and analysed in more detail by Krüger *et al.* (1997) and Strasser *et al.* (1997, 1999). ABS refers to the photons absorbed by the antenna pigments Chl^* . Part

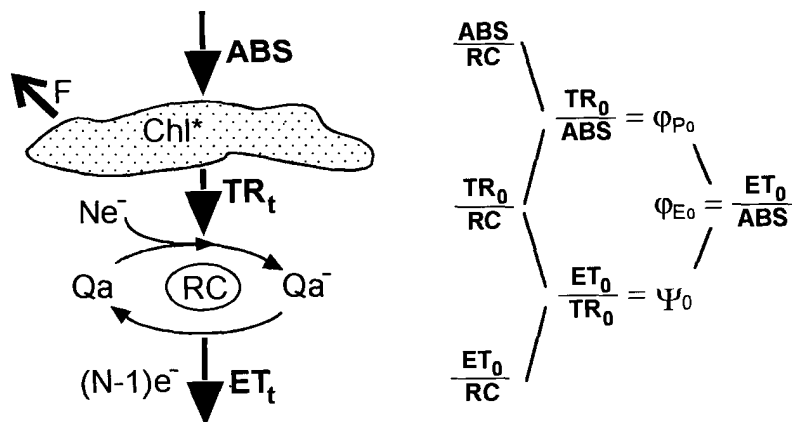


Fig. 1. A highly simplified model of the energy fluxes in a photosynthetic apparatus, as reported in Strasser and Strasser (1995). The specific fluxes (per RC) and the yields are also shown.

of this excitation energy is emitted as fluorescence F and dissipated as heat, and another part is channelled as trapping flux TR to the reaction centre RC . There the excitation energy is converted to redox-energy first by reducing an electron acceptor Q_A to Q_A^- and second by reoxidising Q_A^- to Q_A and creating an electron transport (ET) which finally will maintain the metabolic reactions. The specific energy fluxes at time zero, ABS/RC , TR_0/RC , and ET_0/RC , can be derived from the experiments. The ratio of two such fluxes leads to the quantum yield of primary photochemistry TR_0/ABS , the efficiency that a trapped exciton can move an electron into the electron transport chain ET_0/TR_0 , or the probability that an absorbed photon will move an electron into the electron transport chain ET_0/ABS . The above expressions can be calculated from the experimental values through the formulae summarised in Table 1 (for their derivation see Krüger *et al.* 1997, Strasser *et al.* 1997, 1999).

Results

The experiment: The foraminifers' cultures were kept under alternating dark-light cycles (12 h dark-12 h irradiation of 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$) during several weeks. We chose to conduct the fluorescence measurements every 6 h, because preliminary experiments showed that (a) several hours are needed until the cells reach a steady

Table 1. The formulae for the calculation of the specific fluxes and yields from the fluorescence values, according to the JIP-test.

Specific fluxes	Yields	Yields as ratios of fluxes
$TR_0/RC = (M_0/V_J)$	$\phi_{P_0} = [1 - (F_0/F_M)]$	$\phi_{P_0} = (TR_0/RC)/(ABS/RC)$
$ET_0/RC = (M_0/V_J) (1-V_J)$	$\phi_{E_0} = [1 - (F_0/F_M)] (1-V_J)$	$\phi_{E_0} = (ET_0/RC)/(ABS/RC)$
$ABS/RC = (M_0/V_J)/[1 - (F_0/F_M)]$	$\psi_0 = (1 - V_J)$	$\psi_0 = (ET_0/RC)/(TR_0/RC)$
where $V_J = (F_J - F_0)/(F_M - F_0)$ and $M_0 = 10 (F_{150\mu s} - F_0)/(F_M - F_0)$		

state both in the dark and in the light, and (b) more frequent exposure to the exciting radiation of the PEA-fluorimeter (for more frequent measurements) affects the adapted state. The experiment was conducted during about 7 d, leading to a sequence of 25 measuring periods where the values have been collected. During these 7 d the temperature was slowly elevated from 25 to 32 °C avoiding any unnatural conditions. The cultures were then put back to 25 °C for long-term observations. Between the 19th and 20th measuring periods, high irradiance (HI, 500-550 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was applied during 6 h. Still HI did not exceed the one of a sunny day in the natural environment.

The foraminifer cells tend to move up the wall of the glass tube in the light period, with the consequence that the fluorescence signal measured at the bottom of the tube changes and may even suddenly disappear. Thus, the JIP-test can be used also to monitor the movement of the cells, which depend strongly on several environmental conditions. In order to eliminate the significant fluctuations due to these movements, we used the normalised values of the measured signals over F_0 . Nevertheless, no trend of increase or decrease in F_0 was observed.

The JIP-test was applied at certain stages of the State-1 to State-2 transition, an adaptation process to strong irradiance—here induced by the excitation radiation of the PEA instrument (600 W m^{-2} , about 3200 $\mu\text{mol m}^{-2} \text{s}^{-1}$)—as follows: (A) Every

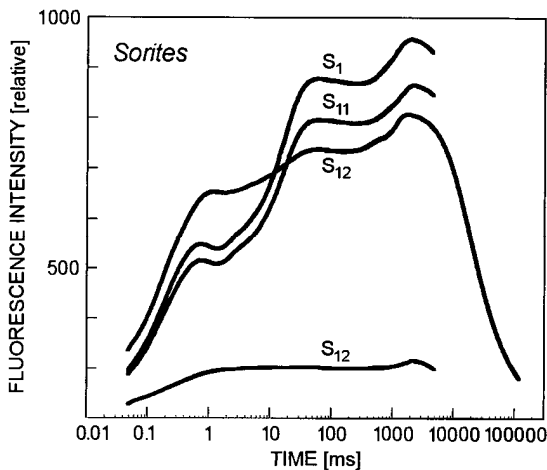


Fig. 2. A set of fluorescence transients recorded at the states S_1 S_{11} S_{12} S_2 of the full State-1 to State-2 transition, here induced by the strong irradiance of the PEA instrument. The transients, presented on a logarithmic time scale, were recorded in *Sorites*. Cultivation conditions: 6 h irradiation (70 $\mu\text{mol m}^{-2} \text{s}^{-1}$), 30 °C.

6 h the organisms were exposed to the excitation radiation for 5 s and measured, giving the values S_1 . (B) Every 6 h the organisms were exposed to the following regime: 1 min dark, 5 s irradiation giving the values S_{11} , 1 min dark, 2 min irradiation giving the values S_{12} , 1 min dark, 5 s irradiation giving the values S_2 . The 1 min dark interval preceding all measurements was experimentally found to ensure the reopening of all RCs without driving towards the dark-adapted state those samples that were at a light-adapted state. An example of the four transients, S_1 S_{11} S_{12} S_2 , is shown in Fig. 2.

The JIP-test response: All photosynthetic organisms, kept for at least 1 min in darkness before the JIP-test, can well be considered as having all RCs open. The irradiance of the PEA instrument is high enough to provoke, within 1-5 s, the closure of all of them. Thus, the fast fluorescence rise recorded by the JIP-test demonstrates the complex kinetics of the closure of the RCs, from F_0 (all open) at the onset of the excitation radiation to F_M (all closed). The shape of this curve is highly dependent on the physiological conditions of the sample.

Fig. 3 shows, indicatively, the fluorescence transients of the three species, from the dark (D) and the light (L) phase of their cultivation, all cultures being at 30 °C. For clarity reasons the curves were vertically displaced. Depending on the conditions, the steps G-H-I-J-K-O became clear. The step K becomes dominant if the cells suffer under heat stress (results not shown). The transition from O to J reflects mainly photochemical reactions leading to the reduction of the electron acceptor Q_A , while the further transient is strongly affected by the subsequent dark reactions in the

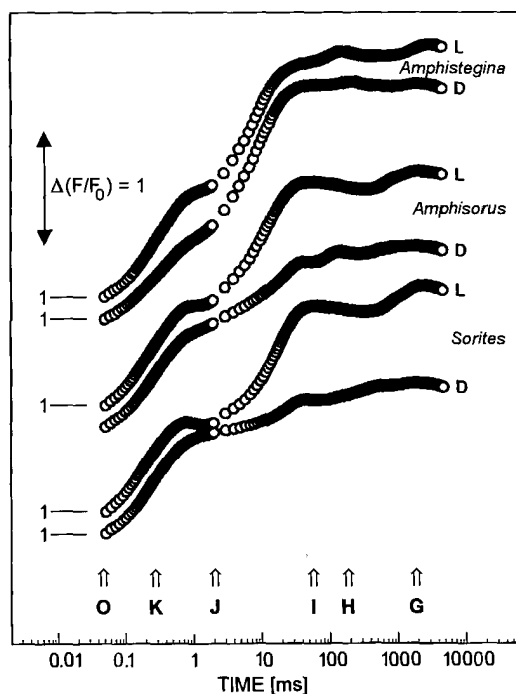


Fig. 3. The typical fluorescence rise curves of the three investigated species on a logarithmic time scale. The fluorescence intensities F_t are normalised over F_0 . For clarity reasons the curves were vertically displaced. The values refer to the S_{11} state and 30 °C cultivation temperature. D stands for the cultures kept for 6 h in the dark and L for those kept for 6 h in the light (70 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

electron transport chain. The three organisms at both phases exhibited a similar O-J rise (Fig. 3), whereas the curves from J to the fluorescence maximum were lower when the cells had been at the dark phase of their cultivation than when they had been at the light one. This down regulation of F_M/F_0 in the dark, which increased with increasing temperature, was more pronounced in *Amphisorus* than in *Amphistegina* and, even more, in *Sorites*.

A principal question is: What is in favour of the organisms, the down regulation or the maintenance of the original behaviour? If we consider the rise of the temperature as a mild stressor which provokes a stress, then we can consider the observed down regulation of many parameters in the dark as an indicator of the deformability of several rate constants. However, deformability and, hence, the deformation induced by a stressor, is the pre-condition for a system to adapt to the new environmental conditions. Therefore, one can forward the hypothesis that organisms which do not react under stress are somehow undeformable and, therefore, unadaptable to stress. However, on the other extreme, there could be the hypothesis that the organism which does not appear to react under stress, is the one who adapts so completely and fast that no change can be observed from outside. Such a behaviour would then be a highly homeostatic one.

The concrete question remains open: Does *Amphistegina*, for example, upon a mild temperature stress show an indeed homeostatic behaviour in respect to the presence or absence of irradiation, or are the cells just unable to down regulate the capacity of their functions in the dark? And a further question: would, for example, *Amphistegina* behave similarly to *Sorites* (Fig. 3) under different environment? The following analysis will concentrate on such questions.

Yields versus temperature and irradiance stress: The different behaviour of *Amphistegina*, *Amphisorus*, and *Sorites* screened by the expressions $\phi_{P_0} = TR_0/ABS$ and $\phi_{E_0} = ET_0/ABS$, is shown (Fig. 4) for the full experimental course of the JIP-test sequences numbered from 1 to 25. Every 6 h the cells were probed by the JIP-test. Therefore, there are always two successive values which refer to the cells kept in the dark (*i.e.*, for 6 and 12 h, denoted as D) or under the irradiance of $70 \mu\text{mol m}^{-2} \text{s}^{-1}$ (*i.e.*, for 6 and 12 h, denoted as L). It was only between the JIP-test sequences 19 and 20, that a 6 h HI was applied instead of the regular irradiation (L).

For *Amphistegina* the light-dark cycles had no effect (sequences 1 to 19) on the two presented expressions, *i.e.*, the quantum yields of excitation energy trapping ϕ_{P_0} and of electron transport ϕ_{E_0} . *Amphisorus* was insensitive in respect to the light-dark cycles concerning ϕ_{P_0} , whereas the expression ϕ_{E_0} showed clear oscillations in phase with the light-dark cycles. *Sorites* showed such oscillations for both expressions. The amplitude of the oscillations increased with increasing temperature. All species reacted in the same way on the HI treatment which stimulated a State-1 to State-2 transition and decreased all quantum yields. However, all samples recovered fully to the same values that they had before the HI treatment. The values shown in Fig. 4 belong to the physiological state S_1 . The more the organisms were driven (by the excitation radiation of the PEA instrument, about $3200 \mu\text{mol m}^{-2} \text{s}^{-1}$) away from the steady state S_1 the more the light-dark oscillations were amplified, to decrease,

however, again when the sample reached the state S_2 which corresponds to the steady state under the strong radiation of the PEA instrument. Even *Amphistegina* and *Amphisorus* showed such oscillations, in all expressions, when the cells were analysed in the S_{12} and even in the S_{11} state (values not shown).

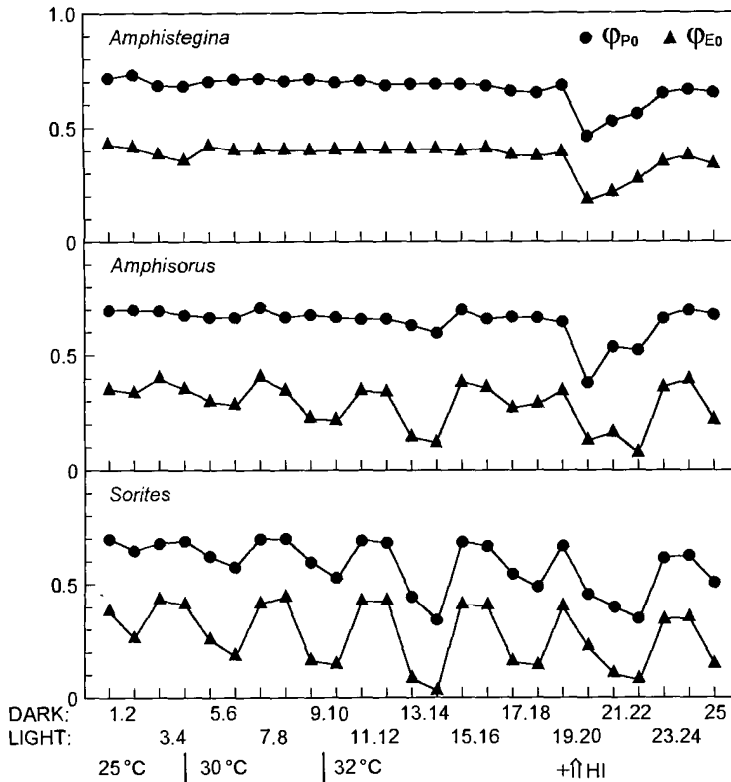


Fig. 4. The quantum yields of the excitation energy trapping $\Phi_{P0} = TR_0/ABS$ and of electron transport $\Phi_{E0} = ET_0/ABS$ for the three studied organisms, during the whole experimental course (samplings 1 to 25, one sampling per 6 h). The presented values refer to the S_1 state. The cultivation conditions, i.e., the temperature, light (L at $70 \mu\text{mol m}^{-2} \text{s}^{-1}$) or dark (D), as well as the 6 h period of high irradiance (HI at $500\text{--}550 \mu\text{mol m}^{-2} \text{s}^{-1}$) are indicated.

Fluxes versus temperature and irradiance stresses: According to the JIP-test one can calculate the specific energy fluxes (per RC) of the organisms at any time of cultivation and for any physiological state during the State-1 to State-2 transition. The specific fluxes at the onset of the excitation radiation, for absorption ABS/RC , trapping TR_0/RC , and electron transport ET_0/RC , are shown in Fig. 5, indicatively for the S_{11} state, and on the same scale for the three organisms. For each species, the values plotted are the mean values from those referring to the same irradiance (i.e., D or L or HI) and temperature (i.e., 25 or 30 or 32 °C) conditions. The x-axis shows, therefore, the trend of temperature and irradiance stresses.

Amphistegina at S_{11} state did nearly not react at all upon the light-dark change. *Amphisorus* oscillated already according to the light-dark cycles and *Sorites* exhibited a much higher response. However, all organisms showed the same trend:

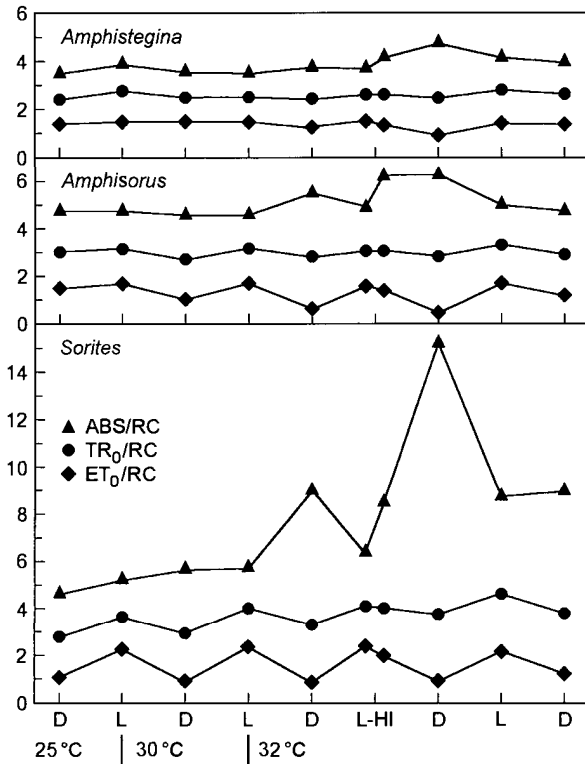


Fig. 5. The specific energy fluxes at time zero, absorption ABS/RC , trapping TR_0/RC , and electron transport ET_0/RC , for the three studied species. The presented data refer to the S_{11} state. For each species, the values plotted are the means referring to the same cultivation temperature and irradiance (D for dark, L for irradiance of $70 \mu\text{mol m}^{-2} \text{s}^{-1}$, HI for high irradiance at $500\text{--}550 \mu\text{mol m}^{-2} \text{s}^{-1}$).

The oscillations of ABS/RC were highly dependent on temperature and HI. The oscillations of ET_0/RC were, in the contrary, nearly insensitive to the temperature rise from 25 to 32 °C. Moreover, they were antiparallel to the oscillations of the ABS/RC . Very small variations were observed concerning the TR_0/RC in all organisms, exhibiting a remarkably constant level with only traces of an oscillating behaviour.

These observations indicate that the organisms tend to keep the trapping flux per RC on a constant level. Mechanisms lead to a homeostasis maintaining a constant excitation rate of the open RCs. This is the best way to avoid over-excitation and photodestruction of the RCs. Such a regulation has been called "cruise control" (Gruszecki *et al.* 1995).

To achieve this goal, the ABS/RC becomes highly variable upon the different stress conditions (temperature and HI). This flux can also be regarded as a measure of the average antenna size which has here a dynamic meaning. It expresses the average number of photons which must be absorbed in order that an exciton excites an open RC. The apparent increase of the antenna size can result from: (a) The increase of the number of Chl molecules per RC. (b) The inactivation of some RCs.

In this case the antennae of these units may also channel a part of their excitation energy to neighbour units over unit-unit energy transfer (cooperativity or grouping, Strasser 1978). The reversible regulation of active and inactive RCs acquires an important physiological meaning. (c) The modification of the rate constants for any excitation energy transfer.

A summary of the experimental results: So far the vitality of the foraminifers was described by introducing several parameters:

- *All experiments are done in time:* The unavoidable parameter is the time over the whole experimental period, here about 7 d. However, the cells were kept under physiological conditions all the time, so that they reached a steady state in all phases of the whole experimental period. Therefore the experimental time can only be used to show the events but it cannot be considered as a free parameter.
- *Environmental parameters:* The irradiances in the light-dark cycles are values of this free parameter. The ambient temperature is a free parameter too.
- *Biological parameters:* The JIP-test measures the fluorescence intensity of the organism during a short time of usually 1 to 5 s. By selecting specific points on the JIP-test time-axis, different biological fluorescence signals are collected and used to calculate the different expressions of the JIP-test. The JIP-test time is therefore a parameter of the internal biological clock which controls the vitality and behaviour of the sample.

Any living system is an open system and has some capabilities to undergo conformational changes to adapt to modified environment. To test this ability to adapt, *e.g.*, to a well defined irradiance (such as the irradiance of the PEA instrument) the sample is irradiated by a precise regime (see "The experiment" under Results, also Fig. 2). In this way we select 4 stages of a full calibrated State-1 to State-2 transition. A continuous state change index can be defined. Here we just use the sequence of the four states S_1 S_{11} S_{12} S_2 as the biological state change parameter.

• *Other parameters* have also been analysed, such as pH, $\text{CO}_2/\text{HCO}_3^-$ (not reported here), or will be analysed, such as UV, pollution, and turbulence of the water.

In Fig. 6 we present the expression F_V/F_0 as a function of irradiation or dark, as a function of temperature, and as a function of the state change parameter indicated as S_1 S_{11} S_{12} S_2 . The values for each species, at each cultivation temperature, and at each state are averaged for the D and L phases of the cultures. The values at the plot denoted as L+HI are the mean values from the HI phase and the preceding low irradiance phase (L), whereas the values denoted as D in the same plot represent the average of the values referring to the dark phase (at 6 and 12 h) that follows the HI phase. All other expressions, as defined by the JIP-test, can be plotted in a similar way. A typical antagonistic effect of irradiance and temperature is clear (see also Havaux and Strasser 1992, Srivastava and Strasser 1996, 1997), since the effect of heat stress is much weaker in the light than in the dark. The optimal temperature can be estimated as the one for which the expression F_V/F_0 has the same value in the L and D phases of the culture. This presents the situation of "undeformability" or high "buffer capacity" in respect to light-dark changes. We see that this situation is achieved at about 25 °C for the Mediterranean *Sorites*, 28 °C for *Amphisorus*, and

30 °C for *Amphistegina*. These temperatures correspond to the optimal and average temperatures for the organisms in their natural environment.

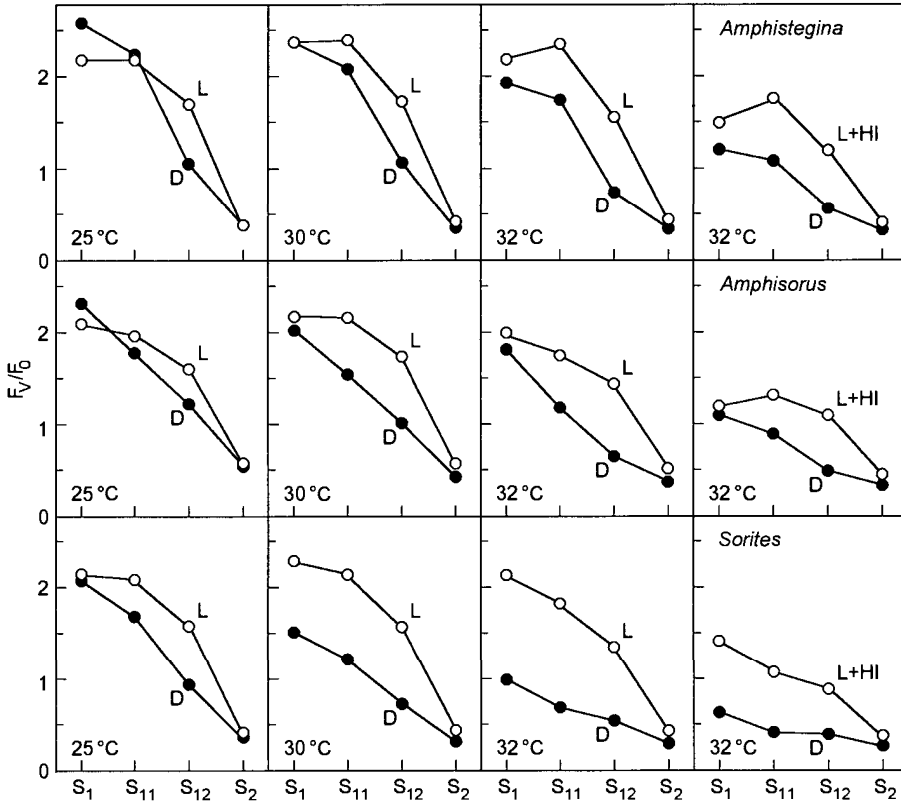


Fig. 6. The extrema of the fluorescence intensities F_0 and F_M are combined in the expression $F_V/F_0 = (F_M/F_0) - 1$ and plotted versus the physiological states S_1 S_{11} S_{12} S_2 . The S_1 to S_2 transition is provoked by the excitation irradiation of the PEA instrument. The values for each species, at each cultivation temperature and at each state, are averaged for the dark (D) and the light (L, at $70 \mu\text{mol m}^{-2} \text{s}^{-1}$) phases of the cultures. The values at the plot denoted as L+HI are the mean values from the high irradiance phase (HI at $500\text{--}550 \mu\text{mol m}^{-2} \text{s}^{-1}$) and the preceding low irradiance phase (L), whereas the values denoted as D in the same plot represent the average of the values referring to the dark phase (at 6 and 12 h) that follows the HI phase.

A remarkable unbalance or deformability is seen as soon as the temperature rises. This unbalance or deformability or capability to distinguish between light and dark under suboptimal temperatures is highly expressed in *Sorites*. However, all organisms are capable to undergo State-1 to State-2 transition to achieve a well balanced steady state in the light of the PEA instrument, which simulates somehow the strong irradiance at noon. The results demonstrate that *Sorites* shows much higher capabilities to sense subtle temperature and irradiance changes than *Amphisorus*. *Amphistegina* is the less capable to sense environmental changes and may therefore

be unable to react and to adapt to new suboptimal irradiance and temperatures (or other parameters).

Visual examination: At the end of the experiment, most *Amphistegina* cells showed darker than normal last chambers, few even with clump of expelled symbionts at the mouth, and one cell was truly bleached. *Amphisorus* almost retained a usual appearance, while *Sorites* had sometimes patches and/or yellowish colours. Over 95 % of all cells were still fully alive one month after the experiments reported here.

Discussion

Though the term "stress" has been precisely defined in physics (Larcher 1995), in biology it has been given widely differing meanings. Probably due to an extension of the physical meaning, many of them converge in attributing stress to any environmental factor "unfavourable" for the living organism under consideration. In accordance, the ability of the organism to survive the unfavourable factor has been called "stress resistance".

Our approach is, however, different in principle. It is focused on the dynamic character of the relation between organism and environment, keeping from the physical approach the concept of *action-reaction*, and offers the possibility for analytical description and quantification (Strasser 1985, 1988, Tsimilli-Michael *et al.* 1996, Krüger *et al.* 1997).

We consider that *stress* has a relative meaning, with the *non-stress* as the reference condition. More precisely, we consider *stress* as a deviation from the *non-stress* situation. The latter is not statically but dynamically defined: it is the situation at which the organism is in *harmony* with its environment or, equivalently, it is at its *optimal state* which can be regarded, based on inferences from nonequilibrium thermodynamics and dissipative structures, as the *state of minimal entropy production*. Any change in the environmental input causes a disturbance of the achieved harmony and leads to *disharmony* with the environment. For the system this situation reflects *suboptimality*. Because of the thermodynamic demand for *optimality*, this suboptimality creates a *state change force* under which the system undergoes *state changes*, i.e., changes in its conformation, until a new constellation of conformational parameters is reached which brings the system to a new optimality. This new optimality is the *attraction point* for the *state change walk* and, once achieved, the state change force vanishes and a new stability, a new harmony with the environment is established. In other words, non-stress is defined as corresponding to thermodynamic optimality, and stress to suboptimality. Accordingly, a *stressor* is every factor which provokes a stress and, thus, leads to suboptimality, while *stress adaptation* is the sequence of processes which realise the state changes leading to a new optimality. Any physical or chemical change occurring during these state changes has been denoted as *strain*.

However, the environmental conditions never cease to manifest alterations and, thus, the system is perpetually undergoing *stress-stress adaptation* processes,

searching and approaching harmony with its environment (Tsimilli-Michael *et al.* 1996).

In this concept, no environmental factor is considered *a priori* as unfavourable and the plant has not to "resist", but it simply reacts. As far as the system manages to adapt, which means that the attraction point is within realistic limits (Strasser 1988), stress is not only harmless but, even more, *constructive* because it results in improved resistance and adaptive evolution. But, if the adaptability of the system is overtaxed, then stress is *destructive*, leading to permanent damages or even to death, as stated by Larcher (1987).

The environmental changes can provoke different responses of an organism depending on its readiness to react, determined by the constellation of structural and functional parameters. It may exhibit a relative stability in its behaviour or reveal an even wide variability. However, in both cases the organism does undergo a shift to suboptimality, being forced to increase the entropy production either to maintain the established state or to search a new state in harmony with the altered environment. Coral reef bleaching is a complex phenomenon triggered by several stress factors. A stress concept based on thermodynamic principles, combined with versatile measuring techniques, may help to quantify the global impact of bleaching.

The reported values show that commercially available instruments are fully suitable to measure and characterise many vital functions of coral reef and temperate foraminifers. Even single cells or small patches of tissues can be measured with high precision anywhere, even on the reef under water. The vitality and activity of ecosystems can therefore be screened and quantified directly *in situ* on the reef. This means that the fluorescence signal can be utilised for the *biomonitoring* of the entire system.

Our results show that the foraminifers react already very typically upon very slight temperature changes in the range where future global changes are expected. The more an ecosystem lives in a constant climate, the less it acquires survival strategies during evolution to respond, react, and adapt to temperature changes. Therefore, *Amphistegina* and, to some extent, *Amphisorus*, may suffer more upon a global temperature rise than *Sorites* which is already used to temperature changes in the Mediterranean sea.

We here propose experimental tests which may be used to screen big ecosystems, in respect to the photosynthetic behaviour of the symbionts, and which may help to understand the bleaching mechanism on a global scale. As a working hypothesis we forward the following concept: A global temperature rise of 1 to 2 °C brings ecosystems already in a measurable suboptimality and, therefore, into an increased sensitivity to other parameters, such as, *e.g.*, the irradiance and/or the increase of pH or the $\text{CO}_2/\text{HCO}_3^-$ concentration. Such questions are worthwhile to be investigated.

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