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VITALITY AND STRESS ADAPTATION OF THE SYMBIONTS OF CORAL REEF AND TEMPERATE FORAMINIFERS PROBED *IN HOSPITE* BY THE FLUORESCENCE KINETICS OJIP

BY

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ABSTRACT

Vitality and stress adaptation of the symbionts of coral reef and temperate foraminifers probed *in hospite* by fast fluorescence kinetics OJIP. - Recent coral reef bleaching, though being global and becoming chronic, is still poorly understood today. Bleaching of corals and large foraminifers involves basically the loss of their photosynthetic symbionts and/or their pigments. Though the cause is unclear, temperature, irradiation and CO_2 are assumed to be primary factors for the symbiosis rupture. In order to establish a monitoring of the vitality of the symbiotic associations, we investigated in three genera of coral reef and temperate foraminifers the behaviour of the photosynthetic apparatus of their symbionts *in hospite*, by means of the JIP-test. By this screening test that we widely use for studies of land plant stress, many samples can be analysed quickly as it needs a measuring time in vivo of only 1 to 5 seconds. We measured the fast polyphasic fluorescence kinetics O-J-I-P of the symbionts by a Plant Efficiency Analyser (PEA) with a 10μs time resolution and 12 bit signal resolution. The measurements can be conducted continuously even on a single cell in a test tube, as well as on the reef. The behaviour of photosystem II of the photosynthetic apparatus, being at different physiological states established by different light conditions and culture temperature, was quantified through a constellation of functional and structural parameters provided by the analysis of the fluorescence transients according to the JIP-test. We moreover induced by strong light, at each physiological state, a State-1 to State-2 transition and again followed the response of the photosynthetic apparatus by the JIP-test. The aim was to investigate whether and how adaptive processes are revealed as regulating the different parameters, in an attempt to get an insight into the capacity of foraminifers for adaptation under different conditions. We observed that the various parameters undergo modifications that differ concerning both their extent and their degree of elasticity, thus indicating that different survival strategies are employed in response to stress. We here witnessed that low light pronouncedly protects the photosynthetic apparatus against the mild warming, and in a lesser extend against the strong light stress, by reducing in some parameters the extent of their deformations and by increasing in others the elasticity of their deformations. The final goal was to correlate adaptability and resistance to bleaching and establish a rapid and easily handled test for the biomonitoring of symbiotic associations *in situ*. This would offer an access to the understanding of the causes of bleaching and possibly serve in foreseeing the future of reefs. A mapping of vitality in terms of performance and behaviour criteria is also proposed, which can serve for the comparison of whole reef ecosystems, or of organisms among an ecosystem, upon any environmental stress and for monitoring the general impact of global changes.

Key-words: coral reef bleaching, foraminifers, fluorescence transient, JIP-test.

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INTRODUCTION

Since the early 80's, massive bleaching affects the reef ecosystem (WILLIAMS & BUNKLEY-WILLIAMS, 1990; GLYNN, 1993; GLYNN, 1996; PÊCHEUX, 1998a). It involves not only corals but also all other cnidarians, as well as molluscs, sponges, ascidians 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 discoloration. Subsequent mortality is highly variable. It occurs world-wide, in all reef biotopes, increasing in magnitude and becoming chronic.

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. Ultra-violets have often been invoked but we found (PÊCHEUX, personal observation) no change of the ozone layer in tropics, nor correlation with bleaching events. Other global factors can be weather changes and general sea water acidification (21% H^+ more) due to CO_2 rise. Whatever the cause is, bleaching demonstrates that the reef ecosystem is near its limits and that small changes of the environmental conditions induce fundamental perturbation of tropical photosynthetic symbioses.

It is not yet clear which of the symbiotic partners is responsible for the rupture of the symbiosis upon temperature elevation, as there are evidences supporting that the host is more susceptible and others pointing out that the photosynthetic symbionts are more affected. However, the response of the photosynthetic symbionts to elevated temperature and its possible implication in coral reef bleaching has been a strong consideration. Chlorophyll a fluorescence measurements, being widely used in studies of land plants stress, were recently applied for corals or their isolated symbionts (IGLESIAS-PRIETO *et al.*, 1992; IGLESIAS-PRIETO, 1995; WARNER *et al.*, 1996). The state of the photosynthetic apparatus appears to be strongly sensitive to bleaching-like conditions and was suggested to be at the origin of the symbiosis rupture. Bleaching has also been more specifically attributed to photoinhibition of photosystem II.

All oxygenic photosynthetic material investigated so far have been found to exhibit upon illumination a fast polyphasic fluorescence rise O-J-I-P during the first second of illumination (for details see STRASSER *et al.*, 1995). A fast screening test, the JIP-test, has been introduced (STRASSER & STRASSER, 1995) for the analysis of the fluorescence transients, based on the Theory of Energy Fluxes in Biomembranes (STRASSER, 1978, 1981; SIRONVAL *et al.*, 1981; STRASSER *et al.*, 1997). By this test, beside various phenomenological parameters directly calculated from the fluorescence values at the steps O, J, I and P, the behaviour of photosystem II (PSII) of the photosynthetic apparatus can be quantified through a constellation of functional and structural parameters (STRASSER & STRASSER, 1995; STRASSER *et al.*, 1996, 1997, 1998). The functional parameters are the energy fluxes for absorption (ABS), trapping (TR) and electron transport (ET); the structural parameters are the flux ratios or yields, i.e. the

maximum quantum yield of primary photochemistry (φ_{P_0}), the efficiency with which a trapped exciton can move an electron into the electron transport chain (ψ_0), and the probability that an absorbed photon will move an electron into the electron transport chain (φ_{E_0}).

As the shape of the fast polyphasic fluorescence transient has been found to be very sensitive to stress caused by changes in different environmental conditions, such as light intensity (TSIMILLI-MICHAEL *et al.*, 1996; SRIVASTAVA & STRASSER, 1997; KRÜGER *et al.*, 1997), temperature (SRIVASTAVA *et al.*, 1997; STRASSER, 1997), drought (VAN RENSBERG *et al.*, 1996), or chemical influences (OUZOUNIDOU *et al.*, 1997), the JIP-test has provided a useful tool for the *in vivo* investigation of the behaviour of the photosynthetic apparatus being at different physiological states and, therefore, for the study of land plant stress.

The first attempt of this study was to investigate whether the JIP-test can be applied as well on whole foraminifers in order to study the physiological state of the photosynthetic apparatus of their symbionts. We here tested three genera of symbiotic large foraminifers particularly ease to laboratory manipulation: *Amphistegina lobifera*, which harbours as symbiont diatoms (*Fragilaria* sp.), *Amphisorus heimprichii* and *Sorites variabilis* who carry the same symbiont as corals (*Symbiodinium* sp.). *Sorites* is a temperate foraminifer, while *Amphistegina* and *Amphisorus* are main components of the reef ecosystem producing a large amount of $CaCO_3$ (LANGER *et al.*, 1997). At least the genera *Amphistegina* is bleaching world-wide (HALLOCK *et al.*, 1995) and appears even more sensitive to this phenomenon than corals themselves. Spectacular shell abnormalities, almost unknown in recent and geological time, are associated with bleaching. However, they are observable in all large foraminifers genera including temperate foraminifers where no bleaching has been detected (PÊCHEUX, 1998b). The certain choice of these three genera for our study was made to serve also the comparison of a coral reef foraminifer with a temperate foraminifer hosting the same symbiont, as well as the comparison of two coral reef foraminifers hosting different symbionts.

All studied species were indeed found to exhibit upon excitation a fast polyphasic fluorescence rise, which could thus be analysed by JIP-test. The shape of the fluorescence transient appeared quite sensitive to several environmental changes tested (e.g. elevated temperature, high light, pH, CO_2). Other foraminifers as well as corals have been also analysed (data not shown). Their behaviour did not principally differ from the behaviour of the organisms reported here.

The final goal is to get an insight into the capacity of foraminifers for adaptation under different conditions, which can further permit a correlation of adaptability and resistance to bleaching. In this frame, we chose here to follow the response of foraminifers to long-term temperature and light changes aiming to investigate whether and how adaptive processes are revealed as regulating different phenomenological, structural and functional parameters of the photosynthetic behaviour of their symbionts.

MATERIALS AND METHODS

Biological samples

Three genera of symbiotic large foraminifers have been used: *Amphistegina lobifera*, *Amphisorus heimprichii* and *Sorites variabilis*. *Amphistegina* and *Amphisorus* were collected in Mauritius in back reef, 1.5 meter depth, and safely transported within one day to the laboratory in Geneva. *Sorites* were collected in the Mediterranean sea near Nice, France, also at 1.5 meter depth, and cultivated during nine months in a closed sea-water aquarium at 25°C under 50-100 $\mu E/m^2s$ 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 experimentation the foraminifers were selected, cleaned and distributed in glass-tubes with 5.5 ml Mediterranean sea-water, daily exchanged with pCO_2-controlled pH at about 8.2. Fifteen parallel tubes for each species were used. The number of specimens per tube was ten for *Amphistegina*, four for *Amphisorus* and one for *Sorites*. The tubes were kept in a thermostated water bath and exposed to light-dark cycles (12 h light at 70 $\mu E/m^2s$ - 12 h dark) over several weeks.

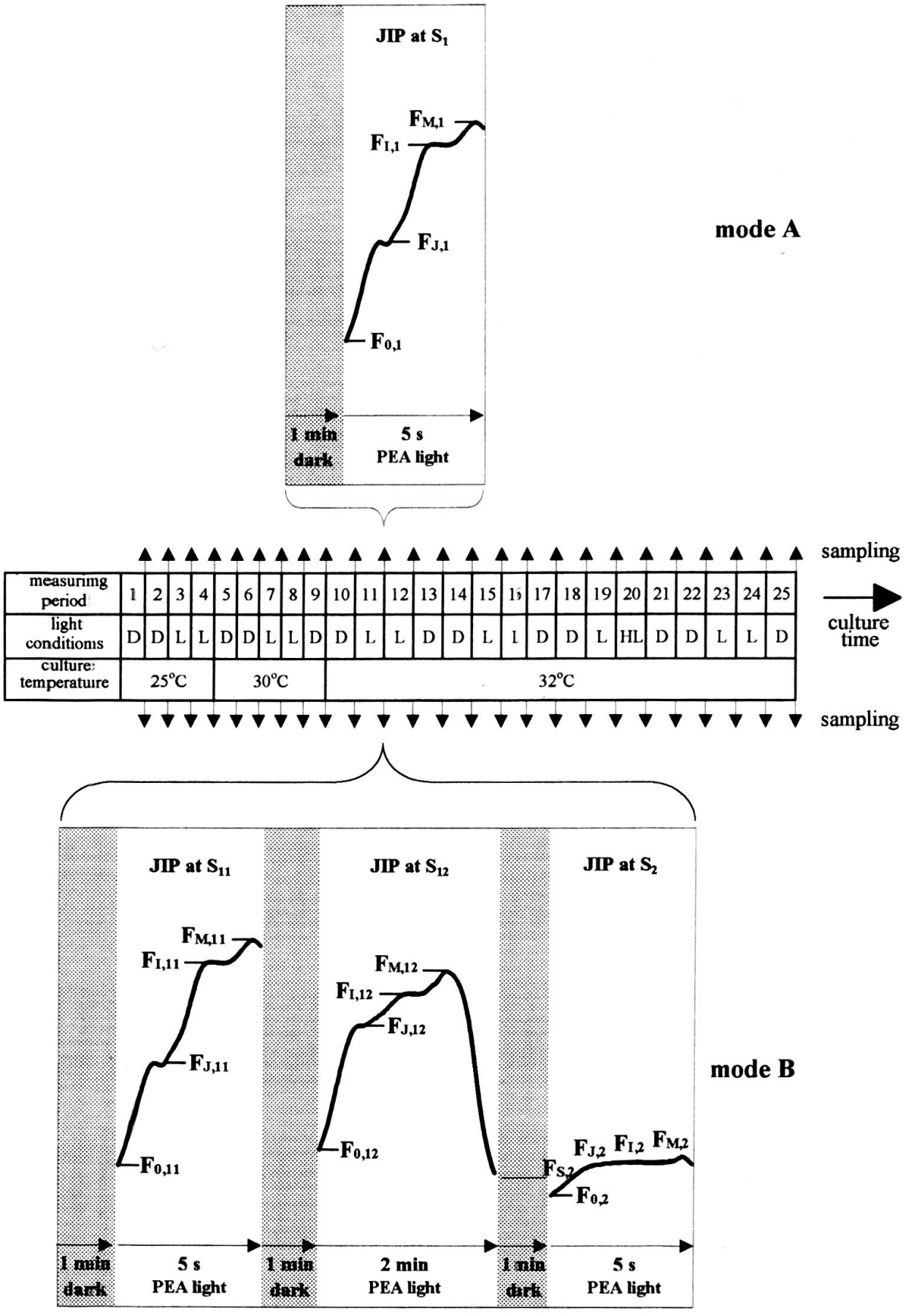
Experimental protocol

Ten tubes for each species were used for the main experiment, while the other five tubes were kept constantly at 25°C and used for preliminary experiments and other observations. The main experiment consisted of a sequence of 25 measuring periods which started 6 hours after the samples had been in the dark and were thereafter conducted every 6 hours. The temperature was gradually elevated from 25°C to 30°C to 32°C avoiding any unnatural conditions. The cultures were then put back to 25°C for long-term observations. During the light phase between the 19^{th} and 20^{th} measuring periods, instead of the light (L) of 70 $\mu E/m^2s$, the cultures were exposed for 6 hours to high light (HL) of 500-550 $\mu E/m^2s$. Though much higher, this intensity is still lower than that of a sunny day in the natural environment. The experimental protocol, showing the light conditions and the temperature of the culture for the six hours preceding the respective measuring period, is presented in Fig. 1.

Fluorescence measurements

Chlorophyll a fluorescence transients of the foraminifer symbionts in hospite were measured by a Plant Efficiency Analyser (PEA, 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 (for details see 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

FIG. 1. The experimental protocol, showing the light conditions and the temperature of the culture for the six hours preceding the respective measuring period. An example of the transients recorded according to the two modes at a certain measuring period is also included, along with the notation of the fluorescence intensities at the different steps which are used by the JIP-test.


JIP at S_1
$F_{M,1}$
$F_{I,1}$
$F_{J,1}$
$F_{0,1}$
1 min dark
5 s PEA light
mode A
sampling
measuring period 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
light conditions D D L L D D L L D D L L D D L L D D L HL D D L L D
culture temperature 25°C 30°C 32°C
culture time
sampling
JIP at S_{11}
JIP at S_{12}
JIP at S_2
$F_{M,11}$
$F_{I,11}$
$F_{J,11}$
$F_{0,11}$
$F_{M,12}$
$F_{I,12}$
$F_{J,12}$
$F_{0,12}$
$F_{J,2}$ $F_{I,2}$ $F_{M,2}$
$F_{S,2}$
$F_{0,2}$
1 min dark
5 s PEA light
1 min dark
2 min PEA light
1 min dark
5 s PEA light
mode B

fluorescence transients were induced by a red light (peak at 650 nm) of 600 W m^{-2} (or about 6000 $\mu E/m^2s$) intensity (excitation intensity) provided by an array of six light-emitting diodes.

The fluorescence measurements for the preliminary experiment were done as following: The test tube was fixed on the measuring head of a PEA-instrument. For the light phases of the dark-light cycles the cells were illuminated with a fibre optic system (light intensity 70 $\mu E/m^2s$) during 55 min followed by 5 min of darkness and 55 min light again. Every hour a fluorescence transient of 5 s was automatically recorded, preceded by 1 min dark (in the light phase) during several days.

The fluorescence measurements for each measuring period of the main experiment were carried out with two different excitation light regimes, denoted hereafter as mode A and mode B. The ten parallel tubes of each species were divided in two groups, each of them measured throughout the experiment by the same mode. For each measuring period, the data of the fluorescence kinetics of the five replica from the same mode were averaged to be used for the calculations. The two modes, leading to different states (for the definition of the states see Results), namely State-1 (S_1), State-11 (S_{11}), State-12 (S_{12}) and State-2 (S_2), are as following:

Mode A: The organisms, after being in the dark for 1 min, were exposed to the excitation light for 5 s and the fluorescence kinetics was measured, giving the data S_1.

Mode B: The organisms were exposed to the following excitation light regime: 1 min dark - 5 s excitation light giving the data S_{11} - 1 min dark - 2 min excitation light giving the data S_{12} - 1 min dark - 5 s excitation light giving the data S_2.

Note that all measurements were preceded by a dark interval of 1 min. This duration of the dark interval was experimentally found to ensure the reopening of all reaction centres (at least the Q_B binding RCs) without driving towards the dark-adapted state those samples that were at a light-adapted state.

In Fig. 1 we present an example of the transients recorded according to the two modes at a certain measuring period, along with the notation of the fluorescence intensities at the steps O-J-I-P(M) which are used by the JIP-test (see below).

The JIP-test

The JIP-test (STRASSER & STRASSER, 1995; STRASSER *et al.*, 1996, 1997, 1998) is here applied to probe the photosynthetic behaviour of the PSII foraminifers symbionts *in hospite* in terms of structure, conformation and function of the PSII of their photosynthetic apparatus. From the fluorescence data stored during the first five seconds, five values were retained as original data and used for the calculation of several phenomenological and biophysical expressions leading to the dynamic description of the photosynthetic samples, being at different physiological states. These values are: the maximal measured fluorescence intensity, F_P, which can here be denoted as 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 to be the fluorescence intensity F_0 when all RCs are open; the fluorescence intensity at 150 μs; the fluorescence

intensity at 2 ms (J step), denoted as F_J; the fluorescence intensity at 60 ms (I-step) denoted as F_I. The fluorescence value at the steady-state $F_{S,2}$ (see Fig. 1) was also retained.

A highly simplified working model of the energy fluxes in PSII is shown in Fig. 2. This model has been elsewhere reported (STRASSER & STRASSER, 1995) and analysed in more details (STRASSER *et al.*, 1997, 1998). The absorption flux ABS refers to the

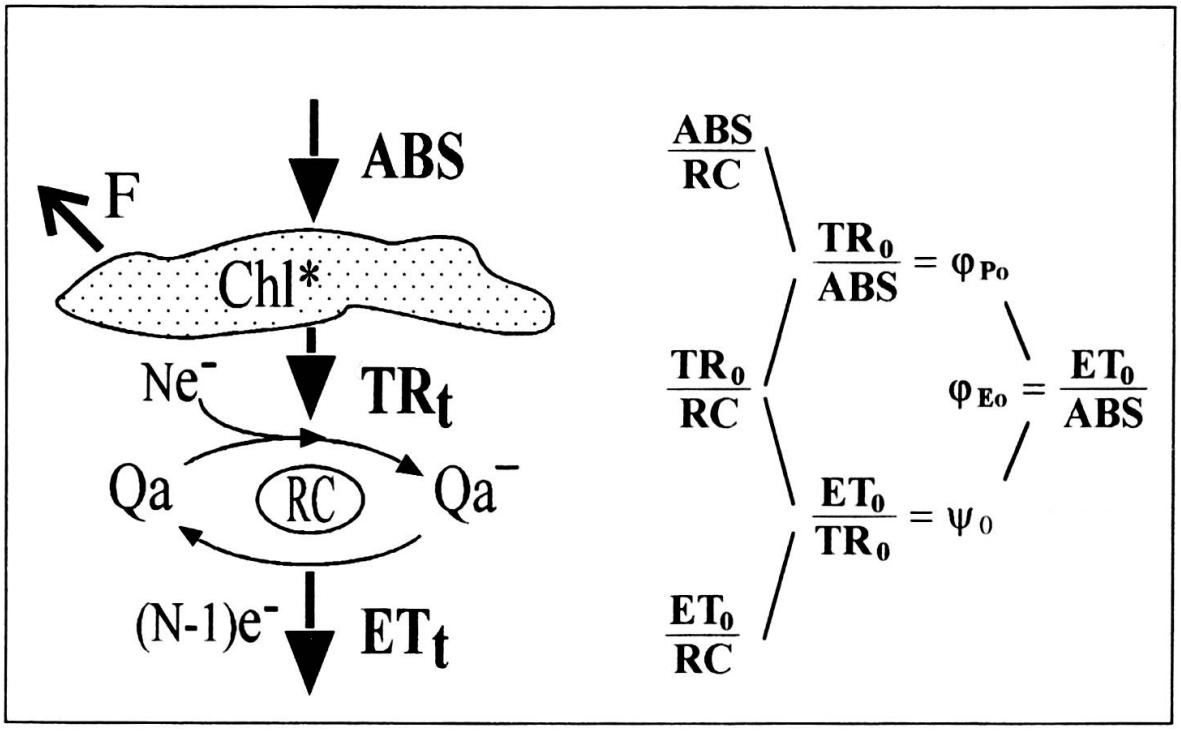


FIG. 2. A highly simplified model of the energy fluxes in a photosynthetic apparatus is shown as it has been elsewhere reported (STRASSER & STRASSER, 1995). The specific fluxes and the different yields or ratios of fluxes are also indicated.

photons absorbed by the antenna pigments Chl*. Part of this excitation energy is dissipated, mainly as heat and less as fluorescence emission F, and another part is channelled as trapping flux TR to the RC. There the excitation energy is converted to redox energy first by reducing the electron acceptor Q_A to Q_A^- which is then reoxidised to Q_A thus creating an electron transport flux ET which will finally maintain the metabolic reactions. From the expressions provided from the JIP-test we here use only the specific energy fluxes (per RC) at time zero, for absorption ABS/RC, trapping TR_0/RC and electron transport ET_0/RC, as well as their ratios, i.e. the quantum yield of primary photochemistry $TR_0/ABS = \varphi_{P_0}$, the efficiency that a trapped exciton can move an electron into the electron transport chain $ET_0/TR_0 = \psi_0$, or the probability that an absorbed photon will move an electron into the electron transport chain $ET_0/ABS = \varphi_{E_0}$.

The above expressions can be calculated from the experimental data through the formulae summarised below. (For the derivation of these formulae see STRASSER et al., 1997, 1998).

specific fluxes	yields	yields as ratios of fluxes
$TR_0/RC = (M_0/V_J)$	$\varphi P_0 = [1 - (F_0/F_M)]$	$\varphi P_0 = (TR_0/RC)/(ABS/RC)$
$ET_0/RC = (M_0/V_J).(1-V_J)$	$\varphi E_0 = [1 - (F_0/F_M)] . (1-V_J)$	$\varphi E_0 = (ET_0/RC)/(ABS/RC)$
$ABS/RC = (M_0/V_J) / [1 - (F_0/F_M)]$	$\psi_0 = (1-VJ)$	$\psi_0 = (ET_0/RC)/(TR_0/RC)$

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

RESULTS AND DISCUSSION

The polyphasic fluorescence rise

Any photosynthetic sample at any physiological state exhibits upon illumination a fast fluorescence rise from an initial fluorescence intensity F_0 to a maximal intensity F_P which depends on the intensity of the illumination and becomes highest under saturating light conditions, denoted then as F_M. Between these two extrema, O and P, the fluorescence intensity F_t was found to show intermediate steps like F_J (at about 2 ms) and F_I (at about 30 ms) while F_M is reached after about 300 ms (STRASSER & GOVINDJEE, 1992; STRASSER *et al.*, 1995). The polyphasic shape of this transient is more clearly revealed when plotted on a logarithmic time scale.

All photosynthetic organisms, when kept for 1 min in darkness, can well be considered as having all their RCs open. The measured F_0 is therefore the fluorescence signal of the photosynthetic membranes with all RCs open, provided that the fluorometer permits the measurement of the initial fluorescence intensity with high precision (as is the case of the shutter-less PEA instrument here used). On the other hand, the light intensity of the PEA-Instrument has been experimentally found to be high enough to provoke, within one second, the closure of all RCs. Thus, the fast fluorescence transient demonstrates the complex kinetics of the closure of the RCs. 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 electron transport chain. The shape of the OJIP-transient is highly dependent on the physiological conditions of the sample and has been found to change its shape according to many environmental conditions. Moreover, an additional step (K) appears and becomes dominant if the cells suffer under heat stress (SRIVASTAVA *et al.*, 1997; STRASSER, 1997).

All foraminifers genera tested in the present work were also found to exhibit this polyphasic fast fluorescence rise upon excitation. In Fig. 3 they are presented, as examples, the fluorescence transients for all three species, both from the dark (D) and the light phase (L) of their cultivation, all cultures being either at 25°C or at 32°C. The

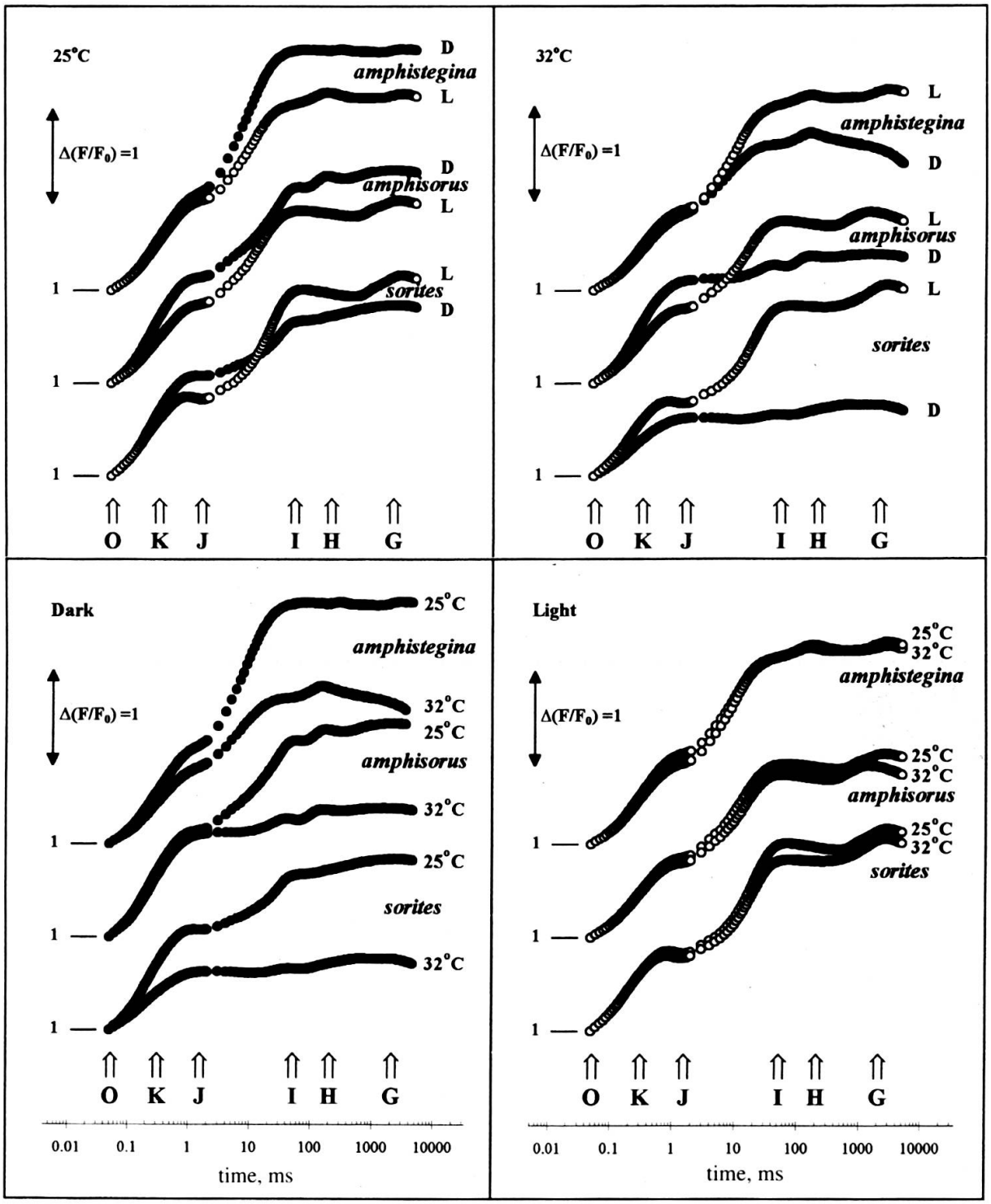


FIG. 3. The fast fluorescence of the three studied species being for 12 h in the dark (D) or in the light of 70 $\mu E/m^2s$ (L), at two cultivation temperatures, 25°C and 32°C. The transients are grouped in two different ways: The upper plots demonstrate the effect of the light conditions at 25°C (left) and 32°C (right); the lower plots show the effect of the temperature when the samples were at the dark (left) or the light phase of their cultivation (right). The transients are presented on a logarithmic time scale, revealing a sequence of steps O-(K)-J-I-H-G.

fluorescence values are normalised on the corresponding F_0 value and, for clarity reasons, the curves are vertically displaced.

It has to be pointed out that the fluorescence transients show here a sequence of more than one steps between the step J and the fluorescence maximum. The fast (300μs) K-step also appears and becomes dominant if the cells suffer under strong heat stress (data not shown). 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. Depending on the experimental conditions, any step can be the highest and would then become the P-step.

Though the intensity of the excitation light permits the closure of all RCs and therefore the P-step represents F_M, we observed that it appears later than in other photosynthetic organisms, especially when the cultures were in the light phase. We therefore extended the measuring time of the fast fluorescence rise to 5 s. The delay affects also the appearance of the I-step which here occurs at 60 ms, instead at 30 ms as in leaves and green algae studied under the same excitation intensity.

The shape and the amplitude of the polyphasic transient depend strongly on the cultivation light and temperature conditions, as shown in Fig. 3. Other species, e.g. the coral *Pavona* sp. and *Stylophora pistillata* as well as the anemone *Anemonia viridis*, were also found to behave in a similar way (data not shown). In order to visualise more clearly the effect of the cultivation conditions we chose to group these transients in two different ways. The upper plots of Fig. 3 demonstrate the effect of the light conditions at 25°C (left) and 32°C (right), while the lower plots show the effect of the temperature when the samples were at the dark phase (left) or the light phase of their cultivation (right).

Both at 25°C and 32°C the shape of the transients between J and G appears quite sensitive to the light conditions, especially in *Amphisorus* and *Sorites* where the steps I and H merge together in the light phase. However, the light conditions have little effect on the amplitude of the fluorescence transients at 25°C. In *Amphistegina* and *Amphisorus* the F_M/F_0 ratio shows a small down regulation in the light phase, while in *Sorites* the down regulation occurs in the dark. On the contrary, at 32°C the F_M/F_0 ratio in all species is lower in the dark than in the light phase of their cultivation, with the difference being much bigger in *Sorites*. However, as demonstrated in the lower plots of Fig. 3, these differences are due to a pronounced down regulation in the dark phase caused by the elevation of the temperature to 32°C, while in the light phase the transients are almost identical at both temperatures.

One JIP-test per hour

In order to follow the response of the three species to changing light and temperature conditions, we had first to choose the timing of the screening measurements. We therefore followed the change of their fluorescence behaviour during the light-dark cycles of their cultivation, with the temperature kept constant at 25°C. During several days a fluorescence transient of 5 sec was automatically recorded every hour (1 min

after light off in the light period, see Materials and Methods), starting from the dark phase. As a phenomenological quantification of the fluorescence transients, the values F_J, F_I and F_M were retained and normalised over F_0 in order to eliminate fluctuations due to the continuous movement of the cells in the glass tubes. Nevertheless, no trend of increase or decrease in F_0 was observed.

In *Amphistegina* and *Amphisorus* the F_M/F_0 ratio showed during the first few measurements in the dark a slight decrease down to the value it retained thereafter in all subsequent measurements both in the light and the dark phases. It thus appears that the difference observed at 25°C between light and dark concerning the F_M/F_0 ratio (Fig. 3, top left) is abolished. The values of F_I/F_0 and F_J/F_0 ratios remained constant as well throughout the course of measurements. It seems that the excitation light used for the fluorescence induction, though illuminating the samples only for a duration of 5 seconds and once in an hour, caused a kind of insensitivity of the samples towards the light conditions of their cultivation. In Sorites the results were quite different. Each subsequent measurement in the first dark phase revealed a further lowering of the F_M/F_0 ratio. Thereafter, the alternating light and dark phases were reflected in a clear oscillation of the three phenomenological parameters F_J/F_0, F_I/F_0 and F_M/F_0, all exhibiting a pronounced down regulation in the dark. It appears that the sensitivity of *Sorites* towards the changes in the light conditions of their cultivation increases due to the excitation light. What is common in all three species is that both the insensitivity of *Amphistegina* and *Amphisorus* and the sensitivity of *Sorites* are due to the decrease of the F_M/F_0 ratio in the first dark phase, and are not related to any change of F_M/F_0 in the light phase.

The patterns of the changes in *Sorites* during two light-dark cycles (not including the first one) are presented in Fig. 4. The duration of 12 hours, both in the dark and the light, was found to permit the normalised fluorescence intensities to level off and full reversibility was observed over many days. However, it needs several hours until the cells reach a steady-state in each phase. In view of this observation and taking into account that the frequent exposure of the samples to the excitation light should be avoided as possible, we chose for the following experiments to carry out the measurements every 6 hours (see Materials and Methods).

Deformation of phenomenological parameters

The down regulation of F_M/F_0 in the dark is an indicator of the deformability of several rate constants. In the frame of the Stress Concept (for a detailed presentation see TSIMILLI-MICHAEL *et al.*, 1996) we consider the capability for deformation as the precondition for a system to adapt to the new environmental conditions. The results so far show that at 25°C *Sorites* acquire this ability only after being sensitised by the excitation light, while *Amphistegina* and *Amphisorus* appear "undeformable" under the same conditions. However, all three species possess this ability when cultivated at 32°C, though at different extent (Fig. 3).

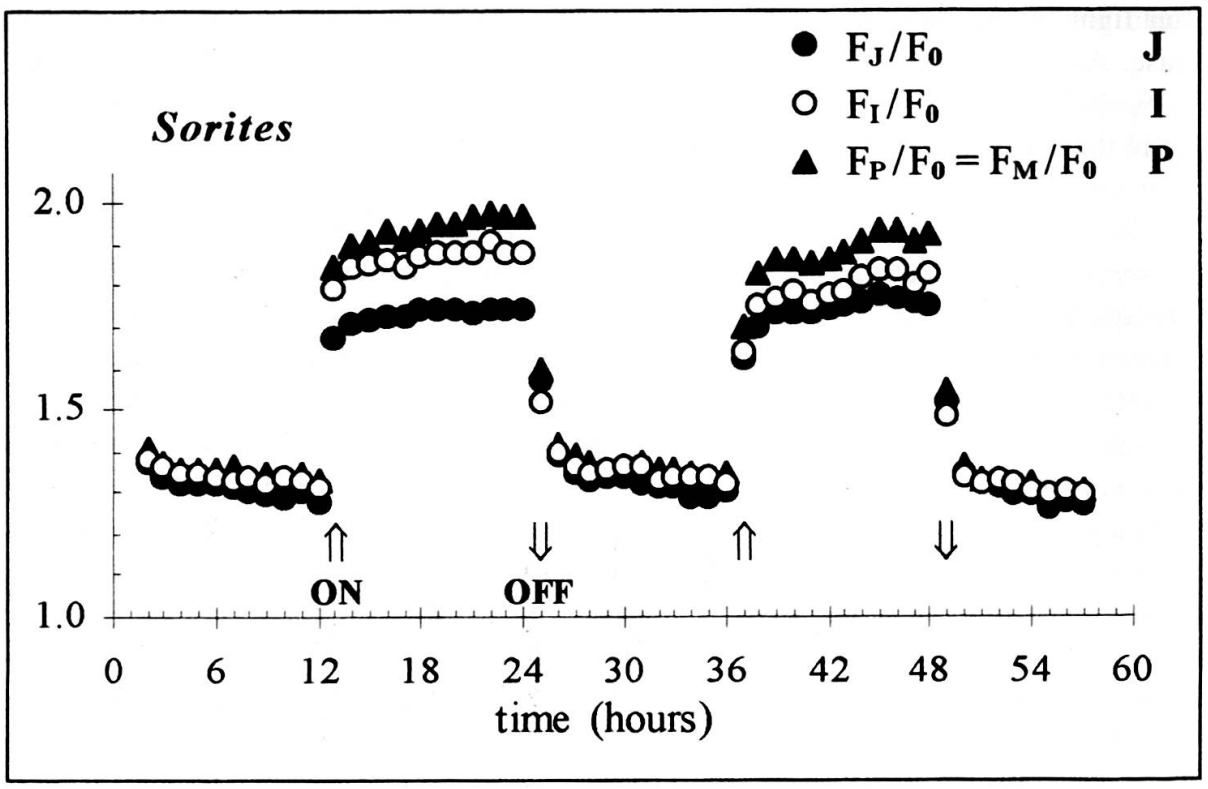


FIG. 4. The pattern of the changes of the phenomenological parameters F_M/F_0 (= F_P/F_0), F_I/F_0 and F_J/F_0 in *Sorites*, during two subsequent light-dark cycles. A fluorescence transient of 5 sec was automatically recorded every hour. For other details see text.

The next step was therefore to follow the response of the three species to the alternating light-dark cycles, under a gradually elevated temperature. In addition we investigated their response to high light, substituting for the second half of a light phase at 32°C (between the 19th and the 20th measurement) the low intensity cultivation light (L) of 70 μE/m^{2}s with a stronger light (HL) of 500-550 μE/m^{2}s.

The results concerning the three phenomenological parameters F_J/F_0, F_I/F_0 and F_M/F_0, are shown in Fig. 5, for the sequence of the 25 measurements. The closed symbols refer to their values at the dark phase (D) of the cultivation and the open symbols to the light phase (L).

The pattern of F_M/F_0 in *Sorites* is pronouncedly different than in the other two species. Already after the second measurement the value of F_M/F_0 starts oscillating and the amplitude increases with the elevation of the temperature. The oscillations are clearly due to a down regulation in the dark, which becomes wider by the elevation of the temperature. It is necessary to remind here that, along with the elevation of the temperature, the increasing number of pre-exposures to the excitation light may also have an effect, as discussed above (see One JIP-test per hour). In *Amphistegina* a decrease of F_M/F_0 is observed in the light phase of the first cycle, which does not thereafter recover and could therefore be attributed to the excitation light. At 30°C the

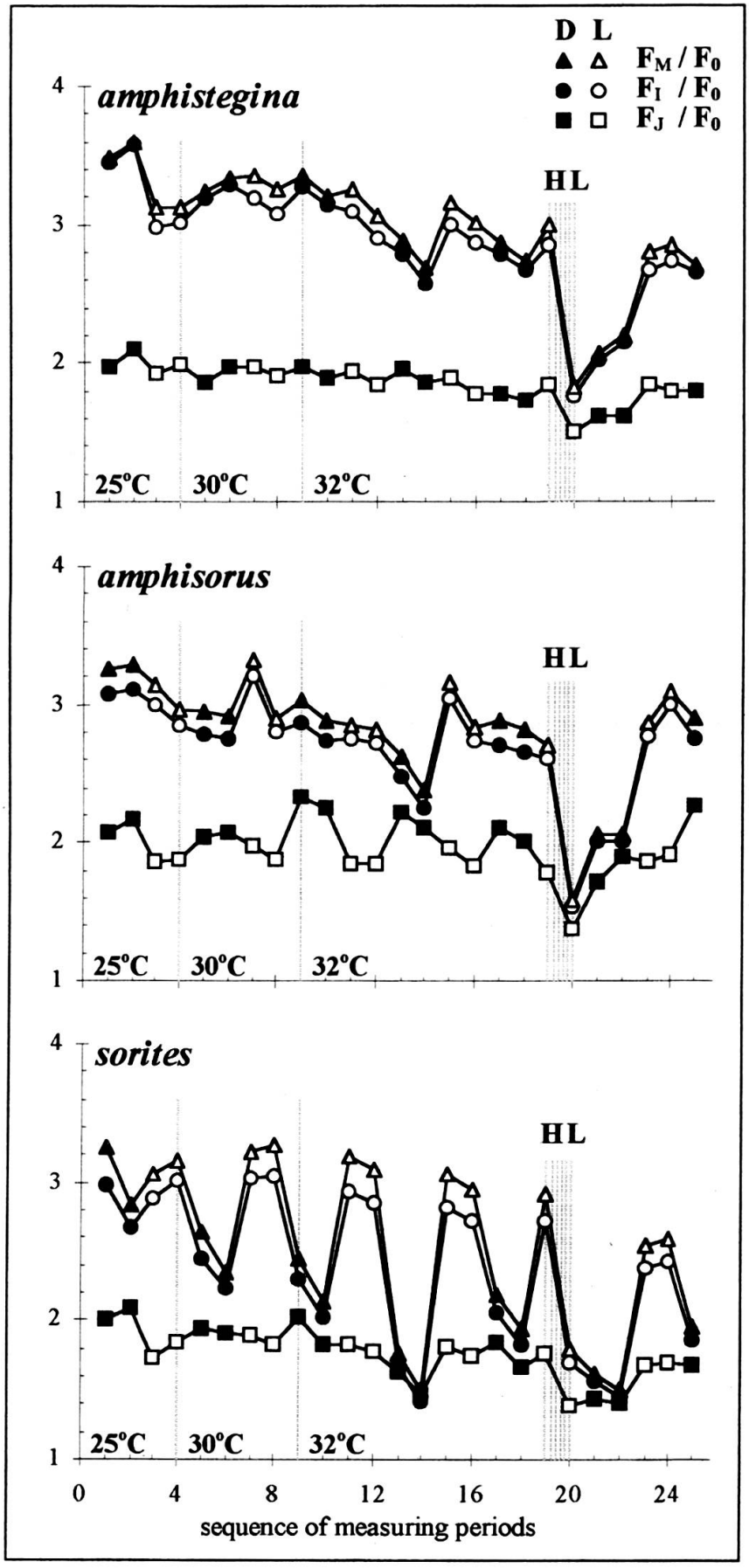


FIG. 5.
The response of the three species, expressed by the F_M / F_0, F_I / F_0 and F_J / F_0 ratios, to the sequence of light-dark cycles, under a gradually elevated cultivation temperature, and for a temporary (6 hours) increase of the light intensity to 500-550 $\mu E/m^2s$ (HL, high light). Closed symbols stand for dark (D), and open symbols for light at 70 $\mu E/m^2s$ (L).

F_M/F_0 ratio appears insensitive to the alternating light conditions, while at 32°C we observe a down regulation of F_M/F_0 in the dark, but much less pronounced than in *Sorites*. A similar trend is seen in *Amphisorus*.

It is worth of pointing out that, though the temperature was already elevated to 32°C before the 10th measurement (6 h D), the effect becomes apparent only at the dark phase (13th and 14th measurements) after the light phase that follows. Even in *Sorites*, where the oscillations appear already at 25°C, the values of F_M/F_0 at this certain dark phase are the lowest. We could assume that, either the F_M/F_0 ratio would decrease after the 10th measurement if the dark phase was further prolonged instead of changing to a light phase, or that it is the combination of temperature and exposure to light that creates the additional stress revealed on the following dark phase by the decrease of the F_M/F_0 ratio.

In general the F_M/F_0 values in the light phases (low light) are rather stable, showing only a slight decrease - more in *Sorites* - upon exposure to 32°C.

The exposure to high light (between the 19th and 20th measurements) strongly decreases the F_M/F_0 ratio at about the same extent in all three species. A partial recovery appears already at the subsequent dark phase both in *Amphistegina* and *Amphisorus* while in *Sorites* the F_M/F_0 ratio continues to decrease and recovers only after the subsequent light phase.

The ratio F_I/F_0 follows closely in all species the F_M/F_0 ratio, while the pattern of the F_J/F_0 ratio appears quite independent. In *Amphistegina* it is completely insensitive to the light-dark cycles, while in *Amphisorus* it shows oscillations with an amplitude which is bigger at 32°C, and with the higher values appearing in the dark phase. In *Sorites*, the F_J/F_0 ratio though not as stable as in *Amphistegina*, does not exhibit any actual oscillations. In all three species F_J/F_0 ratio decreases upon the high light exposure and then recovers completely. By elevating the temperature, the average value of F_J/F_0 decreases slightly in *Amphistegina* and in *Sorites*, and remains more constant in *Amphisorus*.

Deformation of structural and functional parameters

Though the parameters presented so far can well describe and compare the fluorescence transients, they are only phenomenological parameters which can not provide any biophysical or biological information. However, the advantage of screening the samples through such parameters is that, as they are empirical, they do not depend on the theoretical model used.

In order to deduce further information, several structural/functional parameters of PSII can be calculated according to the JIP-test, based on our theoretical model. The formulae are given in Materials and Methods. (For their detailed derivation see STRASSER *et al.*, 1997, 1998).

In Fig. 6 the response of the organisms under study is presented, as probed by structural parameters of their PSII. These parameters are here, the quantum yield of primary photochemistry φ_{Po}, the quantum yield of electron transport φ_{Eo} (or the pro-

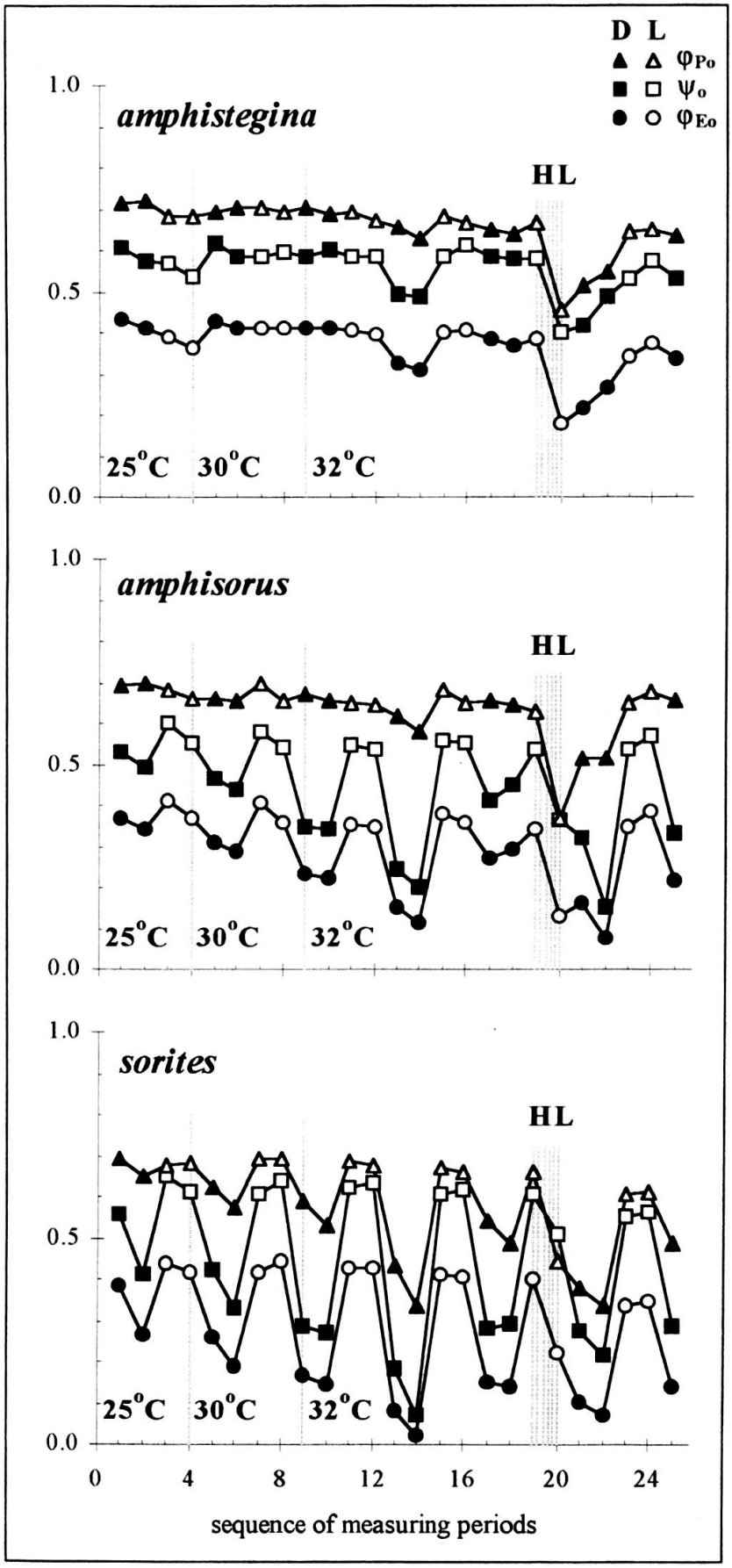


FIG. 6.
The response of the three species to the sequence of light-dark cycles, expressed by the yields (or ratios of fluxes) φ_{Po}, φ_{Eo}, and ψ_o. For other details see legend of Fig. 5.

bability that an absorbed photon will move an electron into the electron transport beyond Q_A^-) and the efficiency that a trapped exciton can move an electron into the electron transport chain ψ_0.

As φ_{Po} is calculated from F_0 and F_M, the changes it undergoes follow those of the F_M/F_0 ratio (Fig. 5) which were above discussed. In *Amphisorus* and *Sorites* the yield ψ_o appears much more sensitive to the cultivation conditions than φ_{Po}, while in *Amphistegina* the patterns of the two yields are quite similar. The wide oscillations that ψ_o exhibits in *Amphisorus* and the even wider in *Sorites* are mainly due to a down regulation in the dark phases which becomes more pronounced when the temperature is elevated from 25°C to 30°C to 32°C, while no trend of increase or decrease is observed at the light phases. It is interesting to note that this regulation, though of different extent, is similar to that of φ_{Po} (or F_M/F_0, see Fig. 5), though the regulation level of ψ_o is completely independent. An additional similarity is observed concerning the effect of HL and the subsequent recovery. Since the changes of φ_{Po} and ψ_0 are in phase, the changes of φ_{Eo}, which depends on both ($\varphi_{Eo} = \varphi_{Po}*\psi_0$), are even more pronounced.

The response of the three organisms concerning functional parameters of their PSII is presented in Fig. 7. These parameters are the specific fluxes (per RC) at the onset of excitation (time zero) for absorption ABS/RC, trapping TR_0/RC and ET_0/RC. It is apparent from first sight that the changes are amplified going from *Amphistegina* to *Amphisorus* to *Sorites*. The patterns of the three parameters can be described and compared on the basis of three criteria: the general trend of the pattern, the response to the light conditions for the different cultivation temperatures and the response to high light.

TR_0/RC shows a highly homeostatic behaviour. In *Amphistegina* it keeps throughout the full course of the measurements a constant value. A small decrease is observed during the dark phases in *Amphisorus* and a slightly bigger in *Sorites*. However, this regulation seems completely unaffected by the temperature elevation, as no trend of increase or decrease is observed neither at the light nor at the dark phases. Moreover TR_0/RC appears not to sense the high light exposure. These observations indicate that the organisms tend to keep the trapping flux per RC on a constant level. It could be speculated that regulation mechanisms lead to a homeostasis which maintains a constant excitation rate of the open RCs, thus avoiding their over-excitation and photodestruction. Such a regulation has been called "cruise control" (GRUSZECKI *et al.*, 1995).

In all species ET_0/RC is more sensitive than TR_0/RC. This is expected since it depends both on TR_0/RC and on ψ_0 ($ET_0/RC = \psi_0*TR_0/RC$) and the latter undergoes deformations of a wider extent and a different trend than TR_0/RC (see Fig. 6). ABS/RC is highly sensitive to the exposure to high light. However, we observe that in Sorites it undergoes much wider deformations (increases) at the dark phases after the elevation of the temperature to 32°C and after the exposure to HL.

It is worth of to focus here on the comparison of the three parameters probing primary photochemistry, i.e. of φ_{Po}, ABS/RC and TR_0/RC. The quantum yield φ_{Po} refers to the whole sample averaging the yield of probably different photosynthetic

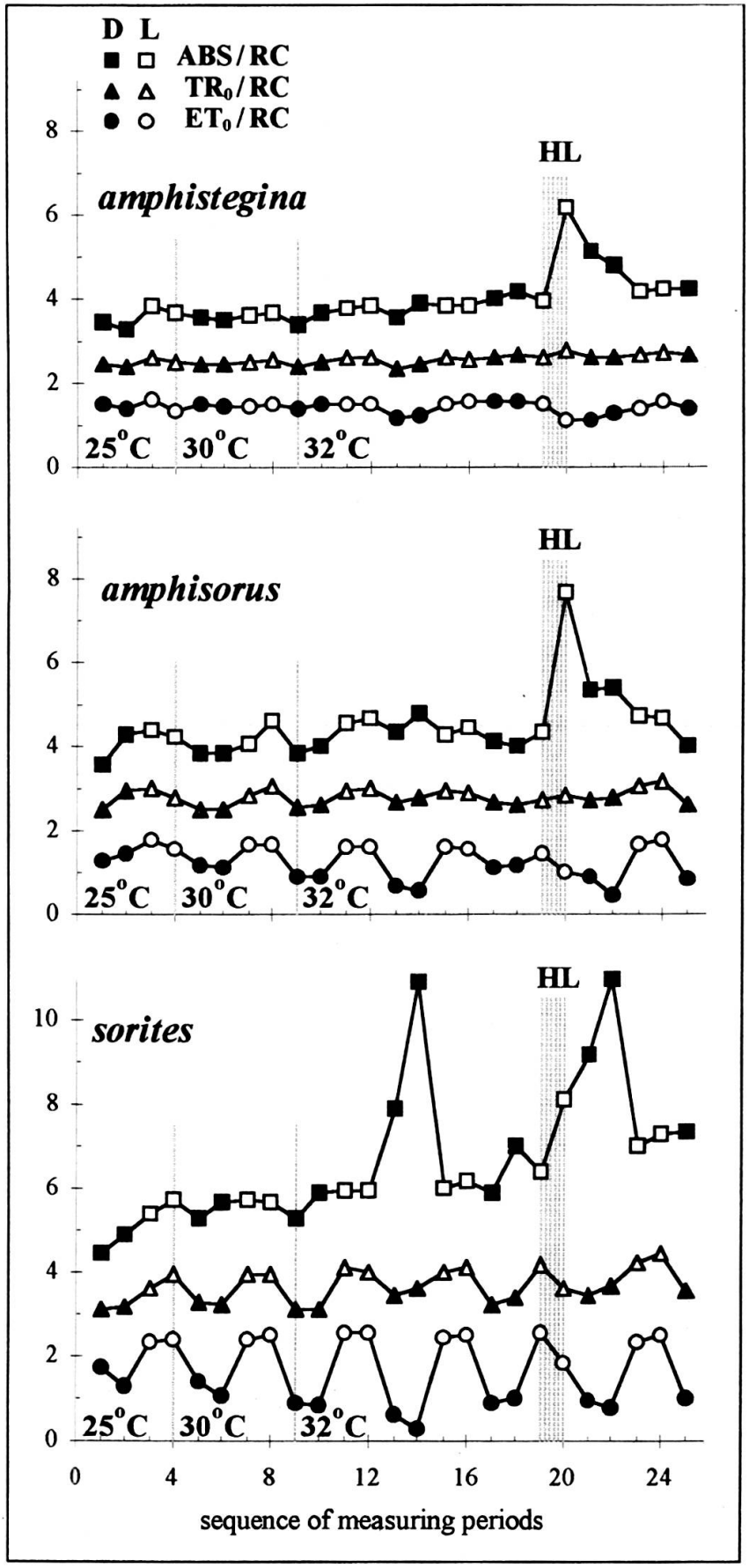


FIG. 7.
The response of the three species to the sequence of light-dark cycles expressed by the specific fluxes ABS/RC, TR_0/RC and ET_0/RC. For other details see legend of Fig. 5.

units. It is possible that an heterogeneity of the sample might arise from the inactivation of some RCs due to their transformation to quenching sinks. However, the JIP-test provides a way to directly calculate TR_0/RC, i.e. the trapping flux per active reaction centre, which is an expression of the photochemical activity of the active RCs. Thereafter, based on the relation $\varphi_{Po} = TR_0/ABS = (TR_0/RC):(ABS/RC)$, the absorption flux per active reaction centre ABS/RC can be calculated. Since we used a constant excitation intensity for the fluorescence induction in our experiments, ABS was proportional to the chlorophyll molecules. Therefore ABS/RC gives also a measure of the average antenna size, i.e. of the total chlorophyll molecules excited per active RC. Hence, an increase of ABS/RC can be regarded as indicating the inactivation of a fraction of RCs (KRÜGER *et al.*, 1997).

If we now use this prism to compare the patterns of φ_{Po}, TR_0/RC and ABS/RC in Figs. 6 & 7, we observe that the effect of the cultivation temperature on φ_{Po}, regardless its different extent in the three species, is associated with changes of ABS/RC, as TR_0/RC is either constant or exhibits oscillations that are unaffected by the temperature regarding both their amplitude and base line. ABS/RC is also responsible in all three species for the pronounced decrease of φ_{Po} caused by the exposure to HL, while TR_0/RC does not appear to sense it. The same is true for the response of *Sorites* to the elevation of the temperature to 32°C, observed temporarily at the second dark phase (13^{th} and 14^{th} measurements). However, the oscillating behaviour of φ_{Po} upon the alternating dark-light cultivation conditions in *Sorites*, is mainly due to the oscillations of TR_0/RC. This can be more clearly observed if one compares the patterns of TR_0/RC in Fig. 7 with the patterns of F_M/F_0 in Fig. 5 instead of those of φ_{Po} in Fig. 6, as F_M/F_0 is more sensitive than φ_{Po} which by definition is scaled between 0 and 1 (mind the relation $\varphi_{Po} = F_V/F_M = 1 - F_0/F_M$).

It is worth of to point out here that, from the response patterns of the three organisms concerning the different parameters, it is apparent that φ_{Po}, which is the parameter commonly used for the description of the photosynthetic behaviour of PSII, is one of the less sensitive in revealing stress effects. This observation favours the utilisation of a test leading to a multiparametric description of the PSII behaviour, especially when the stress applied is not that strong to result in severe inhibition or damage which would then be expressed also by a pronounced decrease of φ_{Po}, which is reported as closely following paling and bleaching.

Indeed, in our study here presented the whole heat treatment does not appear to consist a destructive stress. No loss of photosynthetic pigments was detected, neither any impairment of PSII, as judged from for the F_0 level which, even in the fluorescence transient recorded at the last measuring period was the same as in the one recorded at the first measuring period (data not shown). It must be noted that the heat treatment we used did not exceed the temperature range of the natural habitat of reef foraminifers. However, this is not true for the case of the temperate foraminifer *Sorites* which is living in Mediterranean with a temperature range not exceeding the 30°C. On the other hand, it could be speculated that temperate foraminifers, living in Mediterranean sea and

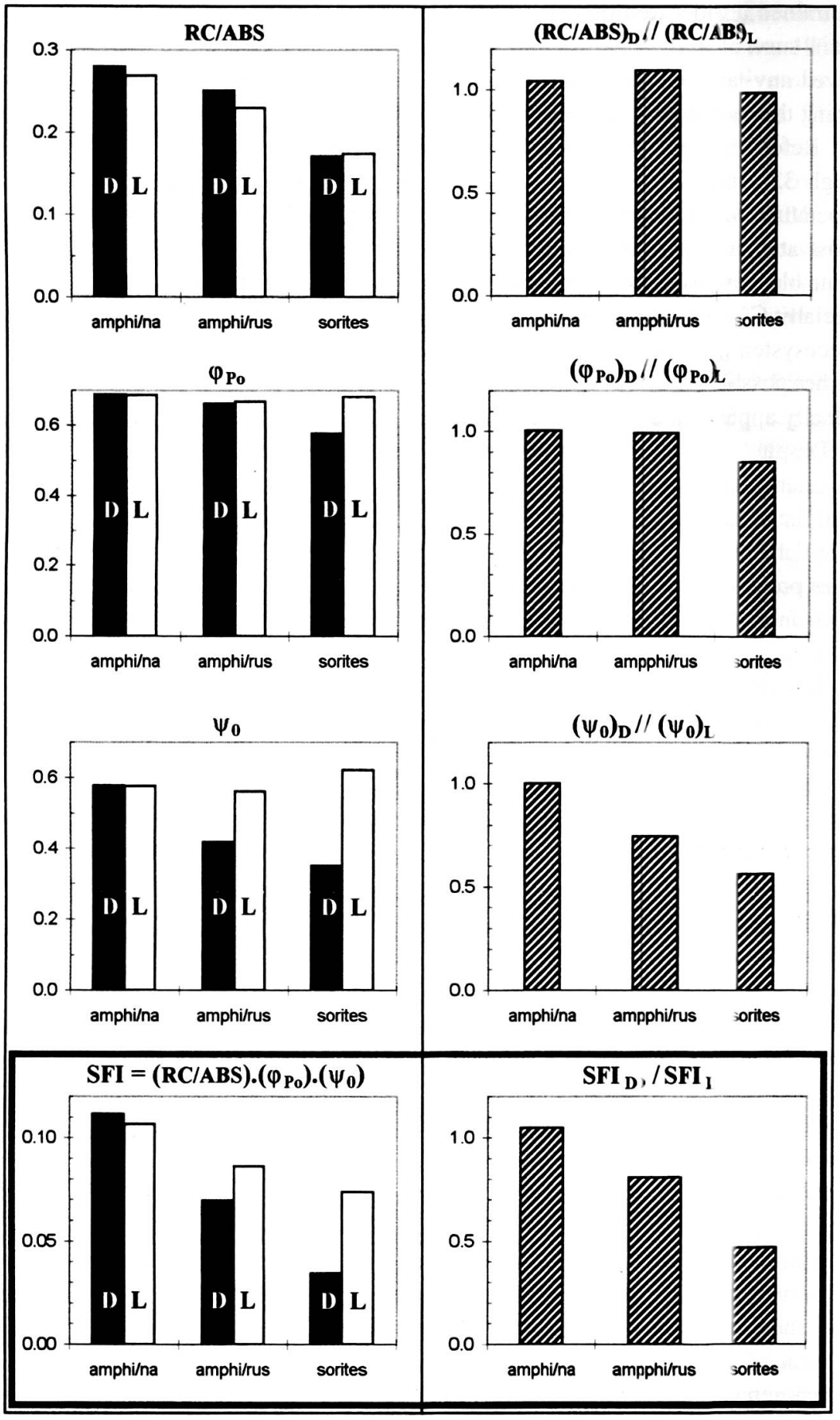
thus trained to wider temperature changes than the species in the tropics, have acquired certain survival strategies to cope with such changes. Indeed, though neither *Sorites* showed any damage during the treatment, the deformations of the several parameters probing their behaviour started already at 25°C.

Referring again to the coral reef foraminifers we studied, it should be noted that though 32°C does not exceed the temperature range of their natural habitat, it is a temperature which seems to bring corals and other associated invertebrates in the field almost at their upper thermal physiological limits (COLES *et al.*, 1976), or even to initiate bleaching events. A possible explanation of the discrepancy is that other factors, especially CO_2, act as synergistic stressors in the field, and may become crucial when the ecosystem is at its thermal limits and is, therefore, already driven to suboptimality. Another possible difference is that in our experiments the elevation of temperature was gradually applied, thus consisting a relatively mild warming.

Despite the differences observed among the three organisms under study and the parameters used to probe their photosynthetic behaviour, what our results clearly show is that the elevation of temperature results in deformations only when the organisms are in the dark phase of their cultivation. This means that the low light used in the light phases protects the photosynthetic apparatus against the mild heat stress. This is a well known antagonistic effect (HAVAUX & STRASSER, 1990, 1992; SRIVASTAVA & STRASSER, 1996).

However, it could be postulated that this protection is realised either by preventing the deformations or by reversing them. In our experiments the elevation of temperature, both from 25°C to 30°C and from 30°C to 32°C, occurred when the samples were in the dark phase. Therefore, in the course of events first comes the deforming in the dark phase and then the reversing of the deformations in the light phase. Therefore our results, without excluding the first postulation, favour the second one.

The expression of this low light thermoprotection is of different extent in the three studied organisms and, for each of them, in the several structural and functional parameters used to probe the photosynthetic behaviour of PSII, as revealed by the different response patterns presented and discussed so far. In order to get an overview of these differences for each organism, we chose three independent parameters, namely RC/ABS, φ_{Po} and ψ_0, and averaged for each of them and for the sequence of the alternating light-dark cycles during the temperature elevation (not including the HL, i.e. up to the 19^{th} measurement) the values in the dark and the light phases separately. These average values, which therefore represent the overall effect of the mild heat treatment on each organism concerning each of the three parameters, are shown in Fig. 8 (left) where D stands for dark and L for light. In the same figure (right) the ratios of the values in the dark over those in the light are also included. It can be assumed that this ratio, which compares the "real" effect of the mild heat treatment in the dark with the effect under protection by low light, gives a measure of the effect of the certain heat treatment on the stability of each organism upon the light-dark changes, in respect to the three chosen parameters.


RC/ABS
0.3
0.2
0.1
0.0
D L
amphi/na
amphi/rus
sorites
(RC/ABS)D // (RC/ABS)L
1.0
0.5
0.0
amphi/na
ampphi/rus
sorites
φPo
0.6
0.4
0.2
(φPo)D // (φPo)L
ψ0
(ψ0)D // (ψ0)L
SFI = (RC/ABS).(φPo).(ψ0)
0.10
0.05
0.00
SFI D / SFI I

The product of the values of all three parameters, (RC/ABS)*(φP_o)*(ψ_0), or equivalently, (RC/ABS)*(φE_o), can be used as an expression accumulating the respective responses of each organism. Physically, this expression can be seen as a criterion of the specific performance of the system, in the sense of giving a measure of the average quantum yield for electron transport per average antenna size. Therefore, as it can be considered as representing an index combining functional and structural criteria, we call it here "Structure-Function-Index", SFI.

The values of this index for the three studied organisms, both for the dark and the light conditions of their cultivation, are shown in Fig. 8, along with the ratio SFI_D / SFI_L, which can accordingly serve as an index of the overall behaviour of the organisms.

S_1 to S_2 transition as an adaptation to the excitation light of the PEA-instrument

Every photosynthetic organism undergoes under high light an adaptation process called State-1 to State-2 transition. We here studied this transition in an attempt to get an insight into the capacity of the organisms to undergo conformational changes which we regard as the basis of their adaptability to the changing environmental conditions in general (STRASSER, 1988; TSIMILLI-MICHAEL *et al.*, 1996).

In the experiments presented so far, we can assume that the photosynthetic organisms were at State-1 (denoted here as S_1), even at the light phases of their cultivation as the light used was of a low intensity (70 $\mu E/m^2s$). However, we used also for 6 h at 32°C a certain high light (HL). Still the intensity of this high light (500-550 $\mu E/m^2s$) is lower than that of a sunny day in the natural environment and it can be speculated that it caused only a partial transition towards the State-2 (S_2) induced by strong light.

In order to follow the complete S_1 to S_2 transition and at every light and temperature cultivation condition, we chose to induce it by the excitation light of the PEA instrument, which is of a much higher intensity (600 W/m^2, or about 6000 $\mu E/m^2s$) simulating the high light conditions at noon. We moreover could in this way measure at the same time the fluorescence kinetics. These experiments were conducted on another set of samples, cultivated parallel to the one used for the previous experiments. At each measuring period the samples were exposed to the PEA light as following: 1 min dark - 5 s light - 1 min dark - 2 min light - 1 min dark - 5 sec light (Fig. 1). This light regime is denoted as mode B to distinguish it from mode A which does not disturb S_1 (see also Materials and Methods). The three individual fluorescence kinetics at each measuring period were recorded and analysed by the JIP-test.

In Fig. 9 we present an example of the set of three successive fluorescence transients (denoted as S_{11}, S_{12}, S_2) recorded according to mode B, along with the one

FIG. 8. A comparison of the overall behaviour of the three species, upon the whole course of the heat treatment (not including the HL and the successive measurements), expressed by the independent parameters, RC/ABS, φP_o and ψ_0, and their product. For each of them, the mean value from the dark (D, black columns) and light phases (L, white columns) during the temperature elevation is presented (left). The ratios of the mean values in the dark over those in the light are also included (right)

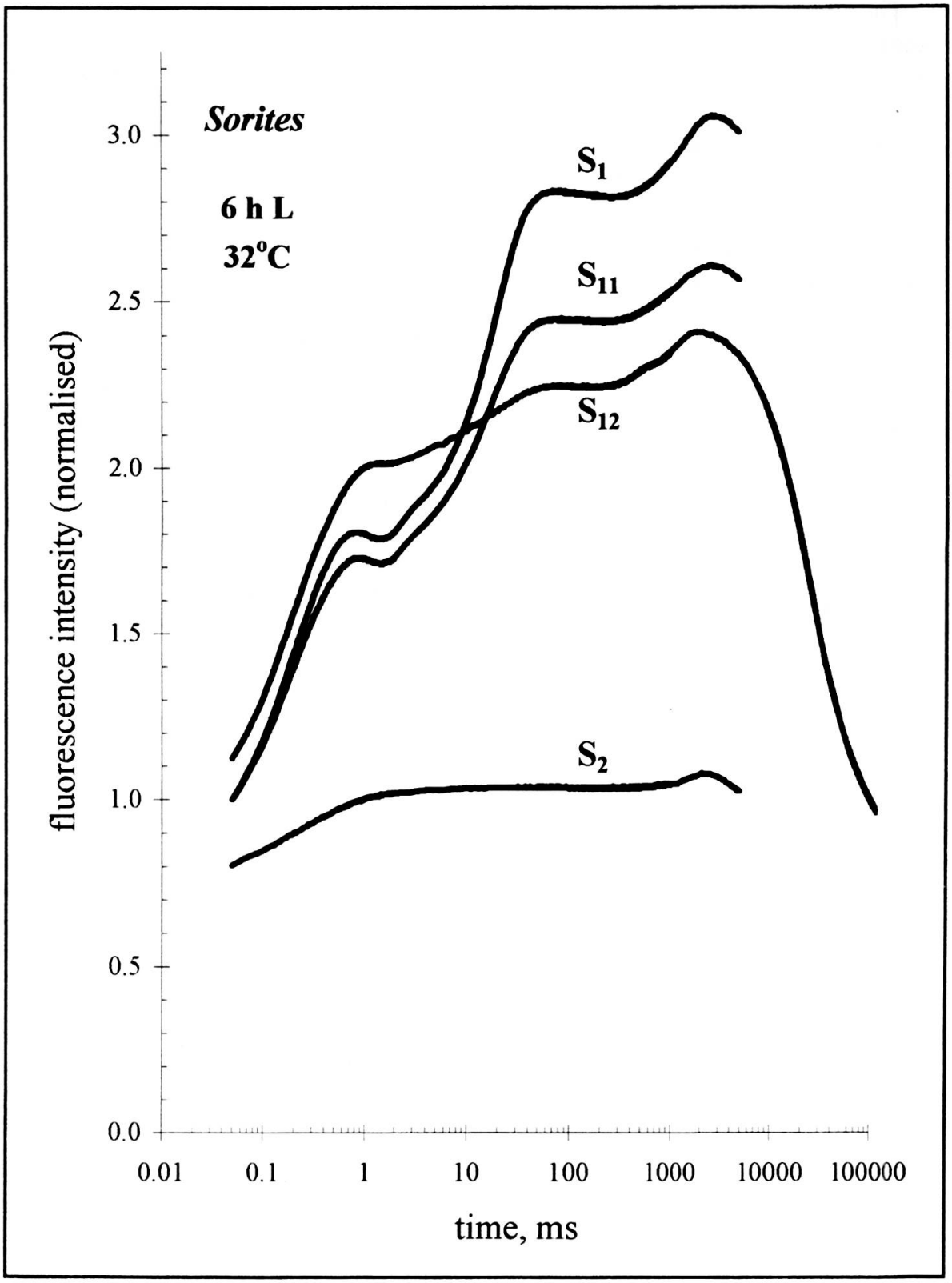


FIG. 9. The set of three successive fluorescence transients (S_{11}, S_{12}, S_2) recorded according to mode B, along with the one recorded according to mode A (S_1), in *Sorites*. The fluorescence values of the kinetics under mode A were normalised over the corresponding F_0 value ($F_{0,1}$), whereas the fluorescence values of all three kinetics under mode B were normalised over the F_0 of the first of them ($F_{0,11}$). The cultivation conditions were 6 h L and 32°C.

recorded according to mode A (denoted as S_1), for the case of Sorites at 6 h L and 32°C (12^{th} measuring period). Since the measurements were not conducted on the same set of samples, the fluorescence values of the kinetics under mode A were normalised over the corresponding F_0 value ($F_{0,1}$), whereas the fluorescence values of all three kinetics under mode B were normalised over the F_0 of the first of them ($F_{0,11}$).

The first fluorescence transient recorded under mode B is already different than that at S_1. In accordance with a previous postulation (KRÜGER *et al.*, 1997) that a sample keeps for a long time the memory of the previous light regimes it has been exposed to, we can as well postulate here that at any measuring period (except the first) the sample "remembers" the preceding S_1 to S_2 transitions it had undergone. We can therefore assume that it is not any more at S_1 but has been driven to a new "state" as an intermediate stage towards S_2. As this drift is a slight one, we here characterised this stage as S_{11} to indicate its proximity to S_1. Comparing now this transient with the subsequent one (Fig. 9) we can observe a further change. Therefore, we can assume that the first 5 second - exposure to the excitation light drives the sample further towards S_2 and, accordingly, we can characterise the new stage as S_{12}. Obviously, it is the initial part of the transient (the fast rise) that refers to S_{12}, while the following slow decrease reflects the completion of the transition which drives the sample to S_2, which is then probed by the third fluorescence transient.

As evident from the example presented in Fig. 9, using the certain light regime for each measuring period, we could select and calibrate 4 stages of a full State-1 to State-2 transition to be further analysed.

The S_1 to S_2 transition as probed by the JIP-test

The phenomenological parameters F_M/F_0, F_I/F_0 and F_J/F_0 presented in Fig. 5 for State-1, were also calculated to screen the behaviour of the photosynthetic organisms under study, being at S_{11}, S_{12} and S_2. The results are shown in Fig. 10, where all three fluorescence values were normalised over the F_0 value of the corresponding transient recorded at S_{11} ($F_{0,11}$). Moreover, the $F_{0,12}/F_{0,11}$ ratio is included in the plots referring to S_{12}, as well as the $F_{0,2}/F_{0,11}$ and $F_{S,2}/F_{0,11}$ ratios in the plots referring to S_2 (for the notations see Fig. 1).

The first to compare is the patterns of the changes at S_{11} (Fig. 10, left column) with the corresponding ones at S_1 (Fig. 5). The most pronounced difference refers to the pattern of F_M/F_0 in *Amphistegina* and *Amphisorus*, closely followed by that of F_I/F_0, which at S_{11} shows oscillations even at 25°C and 30°C. These oscillations are clearly due to a decrease of F_M/F_0 in the dark phases, while only minor differences appear between S_{11} and S_1 concerning the F_M/F_0 values in the light phases. In *Amphistegina* the decrease appears only at 12 h D while in *Amphisorus* it appears both at 6 h D and 12 h D, with the latter more pronounced. Concerning *Sorites*, the oscillations observed at S_1 become amplified at S_{11}, due also to a further decrease of F_M/F_0 in the dark phases. It can be therefore assumed that, in all species being at S_{11}, a certain down

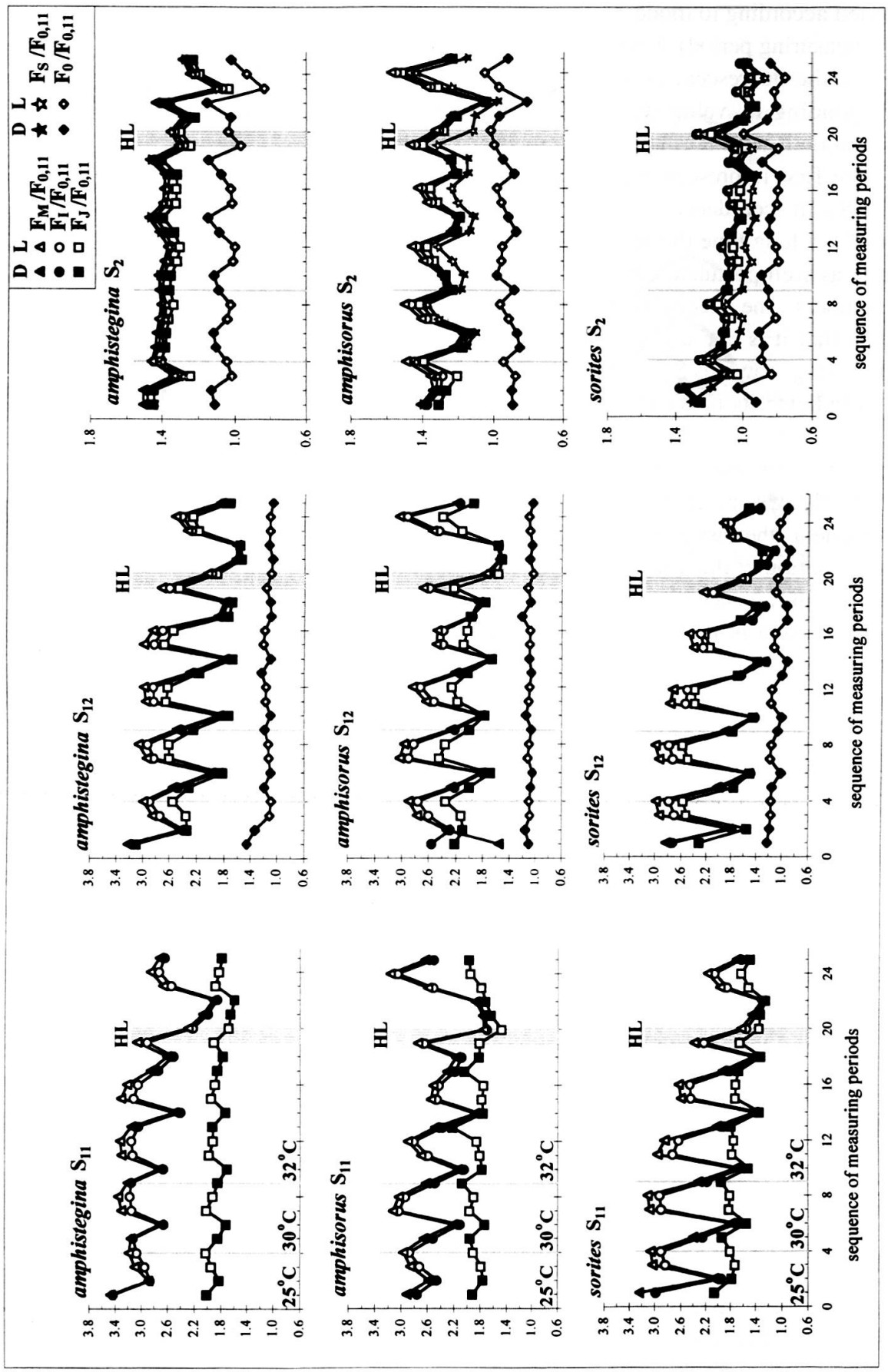


FIG. 10. The response of the three species, being at S_{11}, S_{12} and S_2, to the sequence of light-dark cycles, expressed by the F_M/F_0, F_I/F_0 and F_J/F_0 ratios. The $F_{0,12}/F_{0,11}$ ratio is also included in the plots referring to S_{12}, as well as the $F_{0,2}/F_{0,11}$ and $F_{S,2}/F_{0,11}$, ratios in the plots referring to S_2 (for the notations see Fig. 1). For other details see legend of Fig. 5.

regulation of F_M/F_0 in the dark is superimposed to what takes place when they are at S_1. It could be postulated that this is due to an irreversibility of the deformations that F_M/F_0 had undergone during the S_1 to S_2 transition in the preceding measuring period. This would concomitantly mean that these deformations are plastic in the dark but highly elastic in the light.

The amplitude of the oscillations further increases at S_{12}, however with a simultaneous slight decrease of the values at the light phases (Fig. 10, central column). The biggest difference refers though to the pattern of F_J/F_0 which at S_{12} follows very closely those of F_M/F_0 and F_I/F_0, while at S_{11} it shows traces only of an oscillating behaviour and in a range of values much lower than those of F_M/F_0 and F_I/F_0. The increase of F_J/F_0 towards F_M/F_0 is probably due to the preceding measurement and could be therefore attributed to a difference in the redox state of the electron transport chain, rather than to a state difference. This may also count for the slightly higher than $F_{0,11}$ values of $F_{0,12}$ ($F_{0,12}/F_{0,11} > 1$, see Fig. 10 central column), an observation indicating that a small fraction of RCs could not be reoxidised in the dark interval between the two successive measurements. These RCs could possibly be non-Q_B binding RCs.

At S_2 a wide decrease in all ratios is observed (Fig. 10, right column; note the scale change). However, upon the changes in the light or temperature conditions of cultivation, all the parameters presented show variations which, relatively to the actual values, are not negligible. The decrease of $F_{0,2}/F_{0,11}$ below unity points out that during the S_1 to S_2 transition there occur conformational changes affecting also the F_0 level.

However, the $F_{0,11}$ level at the last measuring period was the same as at the first one (data not shown), as also observed in the experiments dealing with the S_1 state. We can therefore assume that, though the samples measured according to mode B had been exposed (for 24 times) to the additional light stress inducing the S_{11} to S_2 transition and had undergone accordingly much wider changes, no loss of photosynthetic pigments, neither any impairment of PSII was provoked.

The patterns of the yields φ_{Po}, φ_{Eo} and ψ_0 for S_{11}, S_{12} and S_2 are shown in Fig. 11. The difference in the pattern of φ_{Po} between S_{11} and S_1 follows the difference concerning F_M/F_0, which has been above described. However, we observe again that ψ_0, though reflecting different conformational changes, exhibits at S_{11} a parallel to that of φ_{Po} down regulation in the dark phases, however of a bigger extent. We could therefore extend the postulation concerning plasticity in the dark and elasticity in the light for the deformations of ψ_0. As for φ_{Eo}, as it is defined by both φ_{Po} and ψ_0 ($\varphi_{Eo} = \varphi_{Po}*\psi_0$), it shows even wider down regulations in the dark.

The difference of S_{12} from S_{11} concerning the pattern of F_J/F_0 is more clearly revealed in Fig. 11 (central column) by the decrease of the ψ_0 values. It has to be reminded that ψ_0 refers to time zero (at the origin of the fluorescence transient). It is therefore expected that, if at that time the electron transport chain is still partially reduced as above speculated, the efficiency that a trapped exciton can move an electron decreases. The decrease of ψ_0 from S_{11} to S_{12} is an overall decrease that does not specifically affect the light or the dark phases. This overall decrease of ψ_0 is more

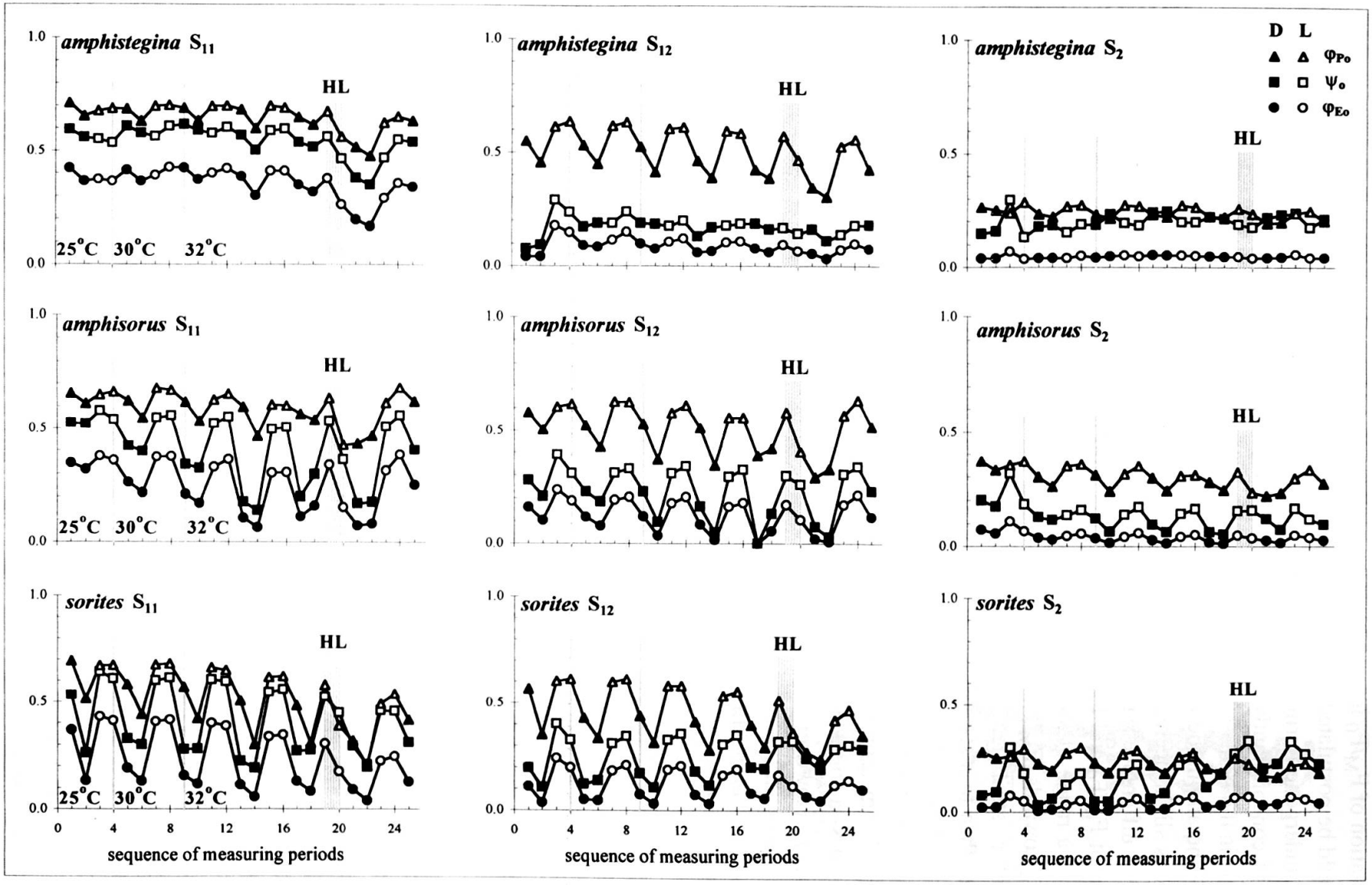


FIG. 11. The response of the three species, being at S_{11}, S_{12} and S_2, to the sequence of light-dark cycles, expressed by the yields (or ratios of fluxes) φ_{Po}, ψ_o, and φ_{Eo}. For other details see legend of Fig. 5.

pronounced in *Amphistegina*. However, in the dark phases of *Amphisorus* and *Sorites* where ψ_0 and, concomitantly φ_{Eo}, were already low when being at S_{11}, the further decrease observed at S_{12} results in very low values which in some measurements are only slightly above zero. By the completion of the transition to S_2 (Fig. 11, right column) a further wide decrease of φ_{Po} is observed, while ψ_0 appears less affected and, in *Amphistegina* even not affected. It is worth mentioning that the oscillations of φ_{Po} at S_2 have a relative amplitude which is comparable to that at S_{11} or even at S_{12}.

In Fig. 12 the behaviour of the three organisms being at S_{11}, S_{12} and S_2 is screened by the specific fluxes ABS/RC, TR_0/RC and ET_0/RC. As in this figure the results referring to the same specific flux are grouped together, the different sensitivity of the three fluxes, indicating different regulations, is clearly visualised. The S_1 to S_2 transition results in a wide increase of ABS/RC, which means that a big fraction of RCs becomes inactivated. This increase is wider at the dark phases. However, at S_{11} we do not observe any difference between the dark and the light phases, except in *Sorites* where the values of ABS/RC are higher at the measuring periods after 12 h D. This means that the deformations caused by S_{11} to S_2 transitions are elastic, both in the light and in the dark, but the light offers some protection against the deformation. Only in *Sorites* the prolongation of dark results in a plasticity of the deformations.

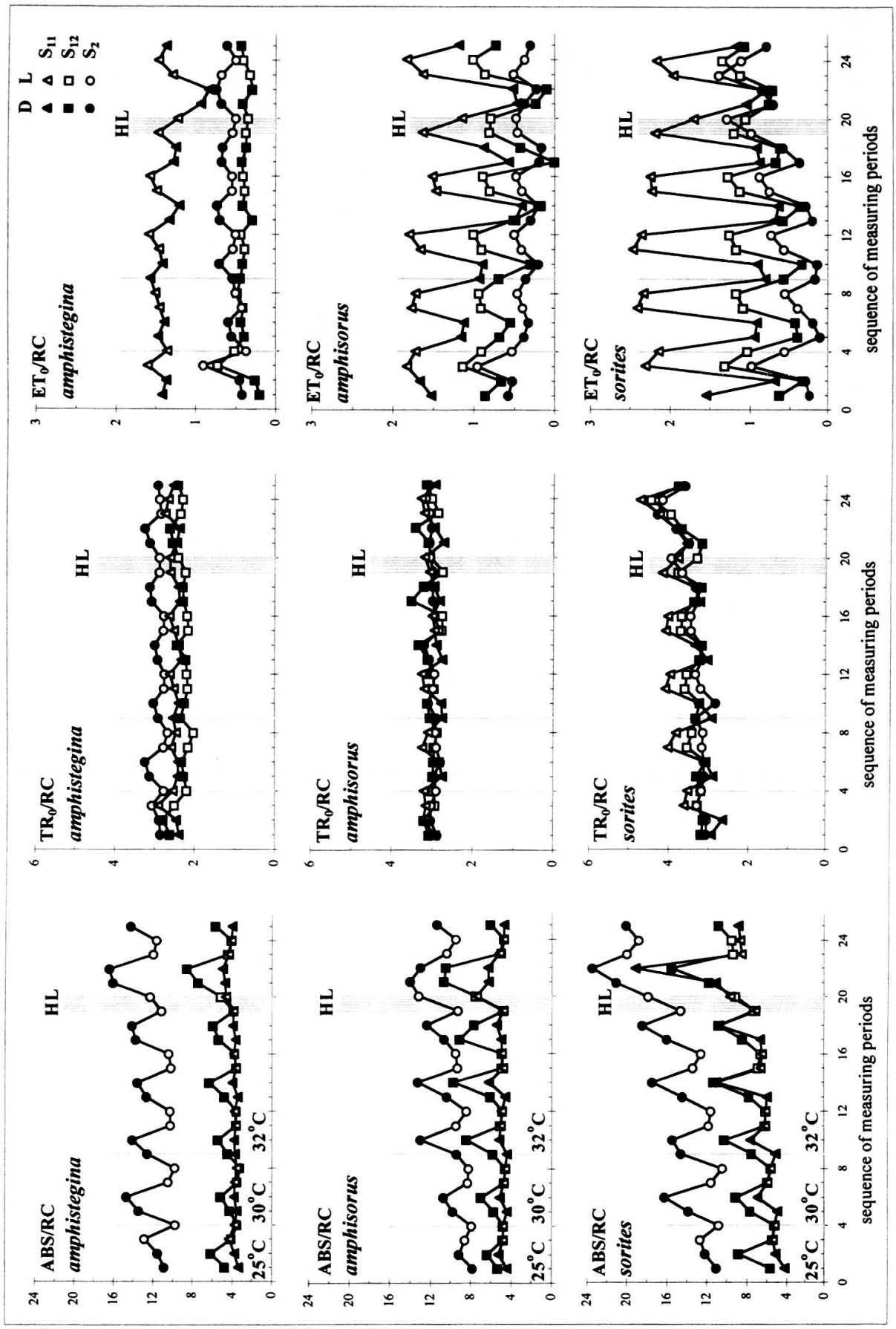
As demonstrated in Fig. 12, TR_0/RC shows a highly homeostatic behaviour. Note also that TR_0/RC is plotted with a scale that, compared to that of ABS/RC, amplifies by four times the perturbations. Such a behaviour was also observed in Fig. 7 upon a mild heat treatment, as well as upon a stress invoked by HL which still was of lower intensity (by one order of magnitude) than the light of the PEA instrument used for the S_1 to S_2 transition.

Concerning ET_0/RC, it is obvious that the oscillating shape of the patterns and the differences between the three states are mainly due to ψ_0 which undergoes much wider changes than TR_0/RC (ET_0/RC = ψ_0 *TR_0/RC).

Over-viewing the experimental results

In our experiments here presented, we were dealing with different kinds of parameters, summarised as following:

- The unavoidable parameter is the time over the whole experimental period, here about 7 days. However, the cells were kept under physiological conditions all the time, so that they reached in all phases of the whole experimental period a steady-state. Therefore, the experimental time can only be used to show the events, as in the figures where the different expressions were plotted versus the sequence of measuring periods, but it cannot be considered as a free parameter.
- The cultivation light conditions, D, L (70 $\mu E/m^2s$), HL (500-550 $\mu E/m^2s$), are values of the light intensity, which is a free parameter.
- The cultivation temperatures, 25°C, 30°C and 32°C are also values of a free parameter.

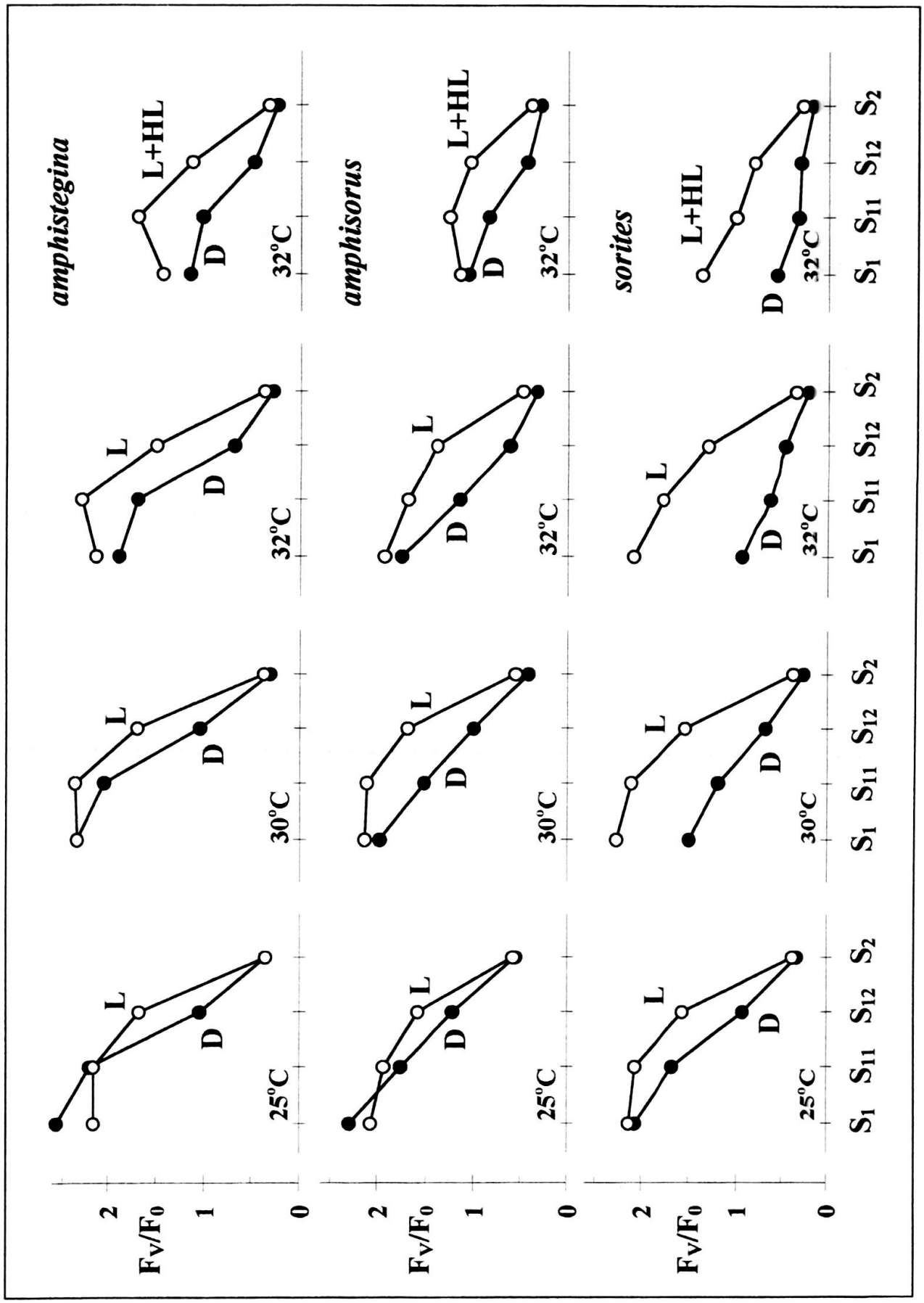

ABS/RC
amphistegina
HL
TR0/RC
amphistegina
HL
ET0/RC
amphistegina
HL
D L
S11
S12
S2
ABS/RC
amphisorus
HL
TR0/RC
amphisorus
HL
ET0/RC
amphisorus
HL
ABS/RC
sorites
HL
TR0/RC
sorites
HL
ET0/RC
sorites
HL
25°C 30°C 32°C
sequence of measuring periods

- The calibrated stages of a full State-1 to State-2 transition, S_1, S_{11}, S_{12}, S_2, are as well values of a free parameter, i.e. of the biological state change induced by the excitation light (about 6000 $\mu E/m^2s$) of the PEA instrument.
- Other parameters have also been analysed, such as pH, CO_2/HCO_3^- (not reported here), or will be analysed, such as UV, pollution and turbulence of the water.
- The different phenomenological, structural and functional parameters derived from the fluorescence transients by the JIP-test are biological parameters describing the photosynthetic behaviour of the symbionts which is at least a basic component of the vitality of the symbiotic association, or even defines it.

In Fig. 13 we demonstrate how the results from such an experimental screening, with a wide range of free parameters, can be summarised to give an overview. We here present as an example the biological parameter F_V/F_0, derived from the extrema of the fluorescence transients F_M and F_0 ($F_V/F_0 = [(F_M-F_0) / F_0]$). All other phenomenological, structural and functional parameters, can be plotted in the same way. The values for each species, at each cultivation temperature, and at each state are averaged for the dark (D) and the light (L) phases of the cultures. The values at the plot denoted as L+HL are the mean values from the high light phase (HL) and the preceding low light phase (L), whereas the values denoted as D in the same plot represent the average of the values referring to the dark phase (at 6h and 12 h) that follows the HL phase.

When in the plots of this figure the values for the light and dark cultures are the same, it means that the effect of the certain temperature both in the dark and in the light is the same, including also the case that the temperature has no effect at all in either phase. We see in Fig. 13 that such a situation of stability upon the light-dark changes (as above discussed in relation to Fig. 8 for other parameters) is achieved at about 25°C for the Mediterranean *Sorites*, 28°C for *Amphisorus* and 30°C for *Amphistegina* when they are at S_1. It could be postulated that the temperatures where this stability is achieved are optimal temperatures. In this frame it is interesting to note that they also correspond to the average temperatures of the natural habitat of the three species. The deviation from stability by the temperature elevation is very wide in *Sorites*, less in *Amphistegina* and almost negligible in *Amphisorus*. Moreover, Fig. 13 shows that as soon as the organisms are driven away from S_1 and towards S_2, the mentioned temperatures are not any more optimal. For *Amphistegina* and *Amphisorus* being at S_{11} the optimal temperatures appear to be at 25°C, while from the trend in Sorites it can be speculated that the optimal temperature would be below 25°C. At S_2 we observe a situation of stability in all species and at all temperatures. However, in this case the apparent stability is due to the strong excitation light of the PEA instrument, upon which the low light offers only a small protection.

FIG. 12. The response of the three species, being at S_{11}, S_{12} and S_2, to the sequence of light-dark cycles, expressed by the specific fluxes ABS/RC, TR_0/RC and ET_0/RC. For other details see legend of Fig. 5.


amphistegina
amphisorus
sorites
L+HL
L
D
F_V/F_0
0
1
2
25°C
30°C
32°C
S_1 S_{11} S_{12} S_2

Visual examination versus photosynthetic behaviour

At the end of the experiment, the cells were visually examined. Most of the *Amphistegina* cells showed darker last chambers than normal. This is what is observed in the field during bleaching events indicating that the expulsion of the symbionts has been initialised. Few of the *Amphistegina* cells showed even a clump of expelled symbionts at the mouth, and one cell was truly bleached. *Amphisorus* almost retained a usual appearance, while some *Sorites* cells had patches and/or a yellowish colour. Comparing the cells studied by the two different excitation light regimes (mode A and mode B) of the experimental procedure, no obvious differences were observed.

After the experiments here reported, the cells were kept in a thermostated water bath at 25°C under the same alternating light-dark cycles and they recovered almost completely. After several months, over 95% of them were still fully alive.

The recovery and survival of the cells appears to be in agreement with our observations from the study of the photosynthetic behaviour of the symbionts and the postulation that no damage of the organisms was occurring upon the course of treatment, but only deformations which were mostly "healed up" by low light.

Therefore, even the visually observed changes in *Amphistegina* cells can be regarded as an indication of the heat stress they had been exposed to, rather than a real and irreversible initialisation of the symbiosis rupture. However, it might also be assumed that, if the stress time was prolonged / or a stronger heat stress was applied / or an additional stressor was applied, the cells, being probably at the edge of their adaptability, would finally bleach and eventually die.

On the other hand, no changes were visually observed in *Amphisorus* cells and very minor in *Sorites*, while the response of the photosynthetic behaviour of their symbionts was more pronounced than in Amphistegina. This is possibly due to the symbiont that *Amphisorus* and *Sorites* host, which is different than that of *Amphistegina*. Comparing *Amphisorus* and *Sorites* that host the same symbiont, the visual examination showing more differences in *Sorites* can be correlated with the higher sensitivity in the photosynthetic behaviour of the symbiont. This must be related, as above discussed, with the fact that the temperature elevation exceeded the range of the natural habitat of *Sorites*. However, it can not be excluded that, despite the minor morphological changes of the *Sorites* cells and the non changes of those of *Amphisorus*, these cells were also at the limits of their adaptability. Indeed, very recently (May 1998) one of us (M. P.) observed that the abundance of *Sorites* in the Mediterranean is highly reduced compared to only a few years before.

FIG. 13. The expression F_V/F_0 versus the cultivation light conditions, the cultivation temperature and the state change parameter S_1, S_{11}, S_{12} and S_2 for the three studied organisms. For details see text.

CONCLUSION: PROBING THE IMPACT OF GLOBAL CHANGES

Coral reef bleaching is a complex phenomenon of high importance. Based on the literature, it appears that this phenomenon is triggered by several stress factors. A working hypothesis can be forwarded, in the frame of a stress concept based on thermodynamic principles (STRASSER, 1985, 1988; TSIMILLI-MICHAEL *et al.*, 1996). A temperature rise of 1 to 2°C can bring ecosystems already in a measurable suboptimality and, therefore, into an increased sensitivity upon changes in other parameters, e.g. light intensity, pH, or CO_2 and HCO_3^- concentrations.

Versatile measuring techniques may help to quantify the global impact of bleaching. We here propose an easily handled experimental test suitable for the measurement and characterisation of many vital functions of coral reef and temperate foraminifers. Even single cells can be measured with high precision on the reef in the water. The JIP-test, by which some hundred samples can be measured per hour in a non-destructive way, allows the derivation of several expressions leading to the dynamic description of a photosynthetic sample at a given physiological state, as we have shown in our experiments here presented. Therefore, the fluorescence signal can be used to monitor the activity and vitality of the entire system directly *in situ* on the reef, thus offering an access to the understanding of the bleaching mechanism on a global scale.

As already for other cases (see e.g. ROMANO *et al.*, 1996), we propose a vitality diagram, shown in Fig. 14, which combines performance and behaviour criteria. The Structure-Function-Index that we introduced and described for Fig. 8, SFI = (RC/ABS)* (φ_{Po}) *(ψ_0), here referring to the dark phases (SFI_D), is plotted versus the corresponding ratio SFI_D/SFI_L (dark over light). The choice of using for this vitality diagram the SFI_D is based on the general conclusion derived from the results in our present study, that the organisms are much more heat stressed in the dark than in the light. This would further indicate that the warm water may be a severe stress factor during the night triggering coral reef bleaching. Even under high light conditions we did not detect any indication of photoinhibition throughout the whole experiment.

In the vitality diagram shown in Fig. 14 an envelope line of the values and the respective mean value is also shown, defining the location of each organism in respect to the different zones of relative vitality. The results presented in this diagram refer to the S_1 state. This means that during the light-dark cycles over several days the samples were measured by the JIP-test for only 5 seconds every 6 hours. Similar diagrams can be plotted for any of the other studied physiological states S_{11}, S_{12} or S_2. Technically, it is a big advantage that already the measurements of 5 seconds at the S_1 state provide the necessary information. Therefore, screening and mapping of big areas become feasible.

Such a mapping can be useful for the comparison of whole reef ecosystems, or of organisms among an ecosystem, upon any environmental stress and can further serve for monitoring the general impact of global changes.

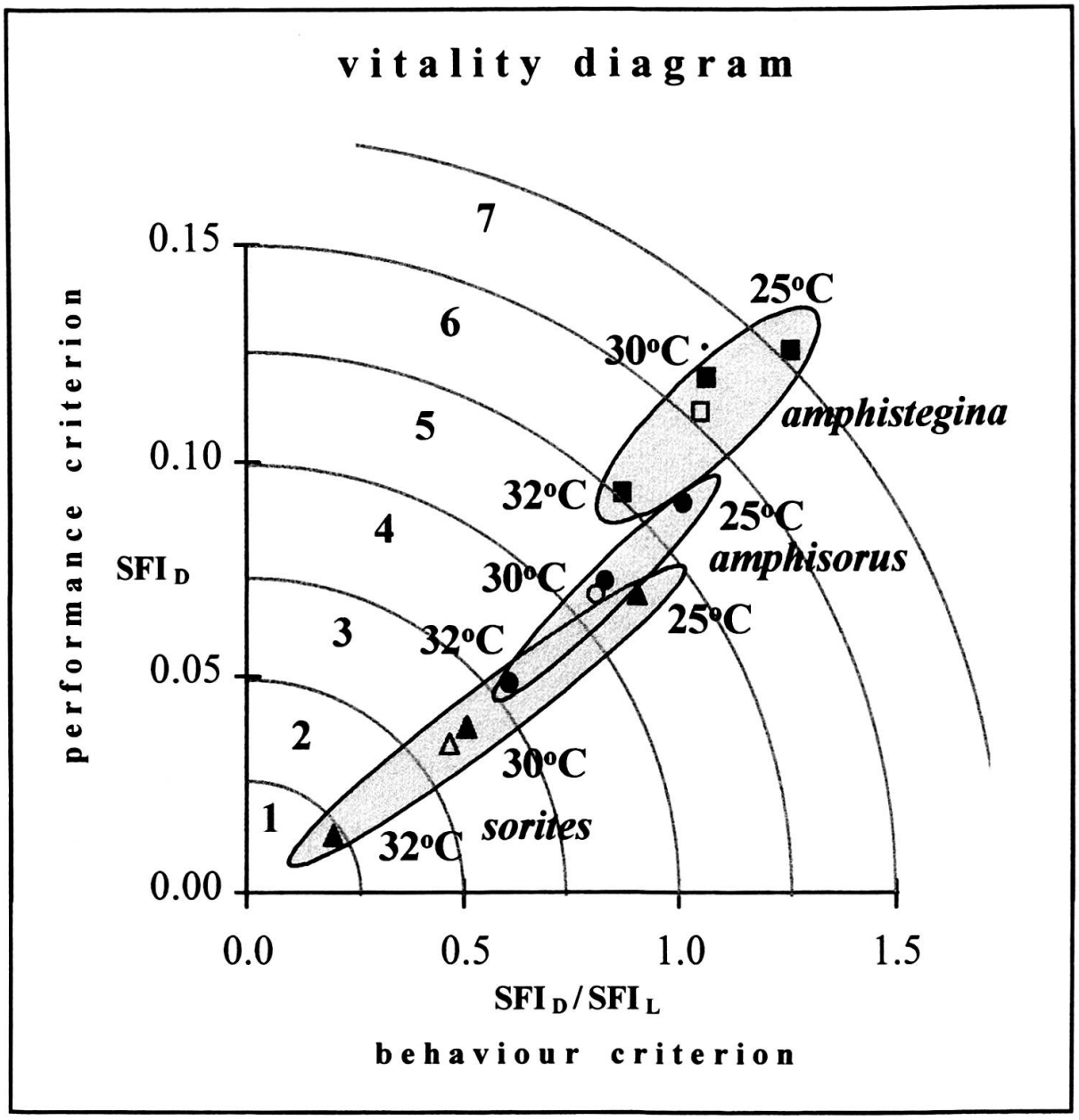


FIG. 14. A vitality diagram in terms of performance (Structure-Function-Index, SFI_D) and behaviour criteria (SFI_D/SFI_L). Squares stand for *Amphistegina*, circles for *Amphisorus* and triangles for *Sorites*. For each value from each organism (all at State-1) the corresponding temperature of the heat treatment is indicated. Open symbols refer accordingly to the average values from the whole course of the heat treatment (as presented in Fig. 8). An envelope line of the values and the respective mean value is also shown, defining the location of each organism in respect to the different zones of relative vitality.

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RÉSUMÉ

VITALITÉ ET ADAPTATION AU STRESS DES SYMBIONTES DES CORAUX ET FORAMINIFÈRES, TESTÉE *IN HOSPITE* PAR LA CINÉTIQUE RAPIDE DE FLUORESCENCE OJIP

Le récent blanchiment des récifs coraliens, bien qu'étant global et devenant chronique, n'est à ce jour que très mal compris. Le blanchiment des coraux ainsi que celui des grands foraminifères est principalement dû à la perte de leur symbiontes photosynthétiques et/ou de leurs pigments. Bien que les causes de ce phénomène ne soient pas claires, la température, l'irradiation et le CO_2 sont supposés être les principaux facteurs de cette rupture de symbiose. Afin d'établir un système d'évaluation de la vitalité des associations symbiotiques, nous avons étudié chez trois genres de récifs coraliens et de foraminifères, le comportement de l'appareil photosynthétique de leurs symbiontes *in situ*, par des mesures biophysiques (JIP-test). Par ce test de criblage, que nous avons déjà largement utilisé pour des études sur le stress des plantes terrestres, un grand nombre d'échantillons peuvent être analysés, car le temps nécessaire pour une mesure *in vivo* est de seulement de 1 à 5 secondes. Nous avons mesuré la montée rapide polyphasique de fluorescence (O-J-I-P) chez les symbiontes par un fluorimètre (PEA: Plant Efficiency Analyser) avec une résolution de 10 µs et une résolution du signal de 12 bits. Les mesures peuvent être effectuées en continu, aussi bien sur une seule cellule dans un tube à essai, que sur les récifs. Le comportement du photosystème II de l'appareil photosynthétique, dépendant étroitement des états physiologiques établis à différentes conditions de lumière et de température, a été quantifié à l'aide d'une constellation de paramètres fonctionnels et structurels provenant de l'analyse des mesures de fluorescence. De plus, nous avons induit par une lumière forte, à chaque stade physiologique, une transition de l'état-1 à l'état-2 et nous avons analysé la réponse de l'appareil photosynthétique par le JIP-test. Le but étant d'étudier si et comment ces processus adaptatifs peuvent être évalués par les différents paramètres mesurés, pour ensuite tenter de mieux comprendre la capacité des foraminifères à s'adapter à différentes conditions. Nous avons observé que la variation des paramètres subit des modifications qui diffèrent pour leur étendue et leur degré d'élasticité ce qui indiquerait que différentes stratégies de survie sont employées comme réponse à un stress. Nous observons qu'une lumière faible protège l'appareil photosynthétique contre un réchauffement et dans une moindre mesure contre un stress dû à une lumière forte. Cette protection est révélée par la réduction de l'amplitude des déformations de certains paramètres et par l'augmentation de l'élasticité d'autres paramètres. Le but final était de corréler l'adaptabilité et la résistance au blanchiment, ainsi que d'établir un test rapide et simple pour une évaluation (biomonitoring) des associations symbiotiques *in situ*. Ceci permettrait de mieux comprendre les causes du blanchiment et pourrait servir à prévoir le devenir des récifs coraliens. Une carte de la vitalité en terme de performance et sur la base de critère de vitalité est également proposé, ce qui pourrait servir pour la

comparaison de l'ensemble de l'écosystème des récifs, ou des organismes d'un écosystème, quel que soit le stress environnemental afin de répertorier l'impact des changements climatiques globaux.

Mots-clés: blanchiment des coraux, foraminifères, cinétique de fluorescence, JIP-test.

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