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## **Coral life as probed by their fluorescence emission**

F Sinniger, R Maldonado-Rodriguez, R J Strasser

*Bioenergetics Laboratory, University of Geneva, CH-1254 Jussy Geneva, Switzerland*  
*Phone : 0041227591944, Fax : 0041227591945, email : Reto.strasser@Bioen.unige.ch*

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### **Introduction**

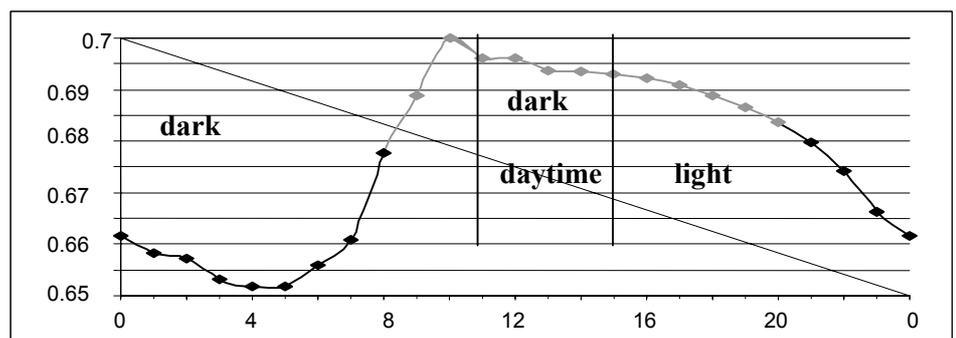
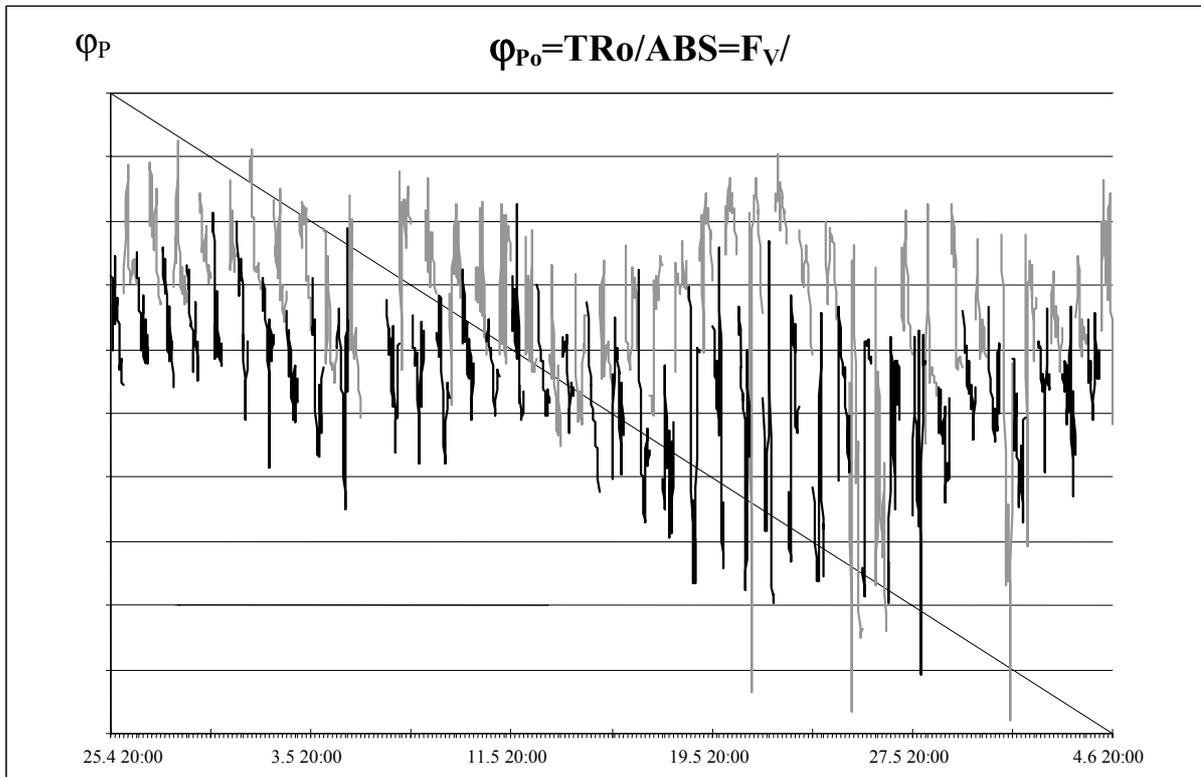
The aim of this study is to develop a method to monitor coral activity by monitoring the photosynthetic activity of their zooxanthellae. The fast fluorescence rise measured with a PEA instrument is used to measure photosynthetic activity without injuring the subject and with some minimal disturbances (i.e. dark adaptation period). The rapidity of the measurement allows the accumulation of many data within short time. The reaction to different stresses (heat, light...) can be rapidly quantified through the interpretation of the fluorescence curve (Tsimilli- Michael and Strasser 2000).

Before looking for the influence of different stresses applied to these organisms, we try first to describe and understand what underlies the cyclic variation observed in during long term monitoring. We compare the results obtained in one polyp of *Actinodiscus sp.* to another *Actinodiscus sp.* and a colony of *Porites porites*. This will give also a basis for comparing stress-induced reactions. The activity of a polyp of *Actinodiscus sp.* was measured each hour during several months. In this period the polyp grew and formed new polyps by budding.

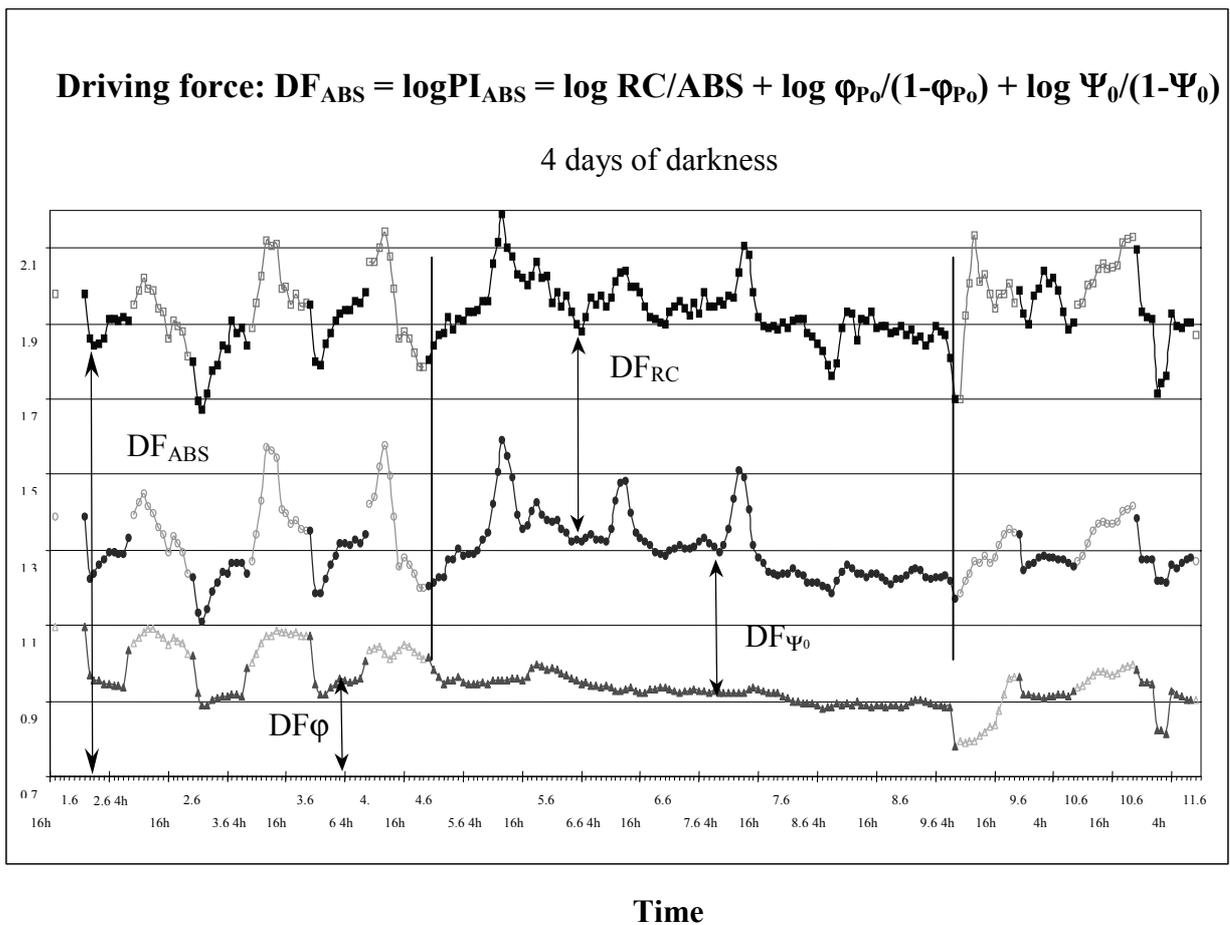
### **Materials and methods**

Corals were grown in a sea water aquarium of 60 l with the polyps living on rocks close to the glass wall. The temperature (24°C) and salinity (s.w. = 1028-1030 kg/m<sup>3</sup>) were kept constant. Illumination was provided by four lamps: three 18 W reef aquarium neon lamps and one blue neon lamp. Chlorophyll *a* fluorescence was induced every hour for 20 seconds (during the first two months of the measurement 10 s pulses were used) with a PEA instrument (Plant Efficiency Analyser built by Hansatech Instruments Ltd, King's Lynn, Norfolk, England) over a period of several months. The 20 s pulse consisted of red light (3500 μmol m<sup>-2</sup> s<sup>-1</sup>).

The aquarium lights were on between 8:00 h and 20:00 h. However, before each measurement the polyps were dark adapted for 12 minutes. Leading to the following protocol: 8:00 h, lights on, 8:45 h, lights off, 8.57 h, 20 s measurement; 9:00 h lights on, etc. till the final lights off at 19:45 h. The first point was measured 50 μs after the onset of the actinic light (F<sub>0</sub>). The measurements were made on a logarithmic time-scale with the first 200 points measured with a 10 ms resolution.

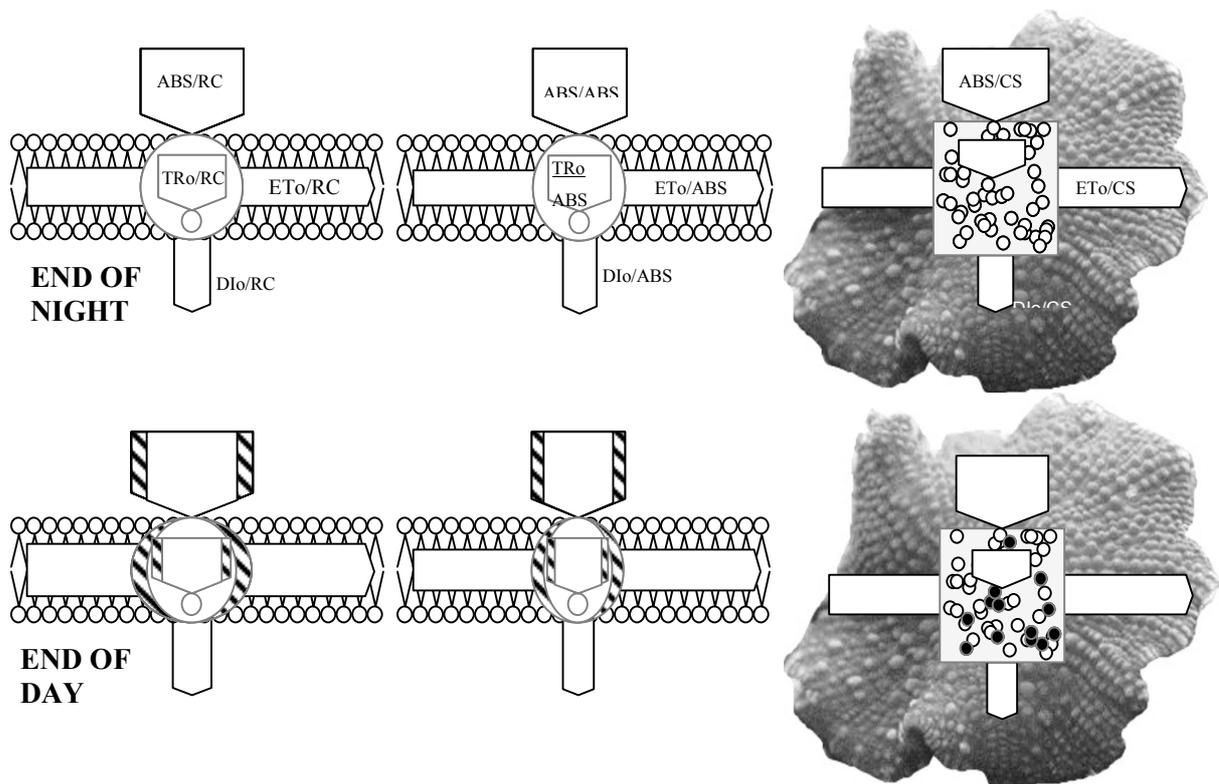


**Fig.1** : Representation of the trapped energy per light absorbed also called  $\phi_{P_0}$ , or maximal quantum efficiency of primary photochemistry measured on a polyp of an *Actinodiscus sp.* The main graph shows the values obtained each hour during 6 weeks. Whereas the insert represents the gliding 3 hour means of the average of the daily cycles of the first 11 days.



**Fig.2** : Photosynthetic driving forces obtained from the measurements of a colony of *Porites porites* during 10 days. After 3 days of 12 h light 12 h dark the coral was kept in darkness for 4 days. Gray parts of the curve represent daytime measurements, whereas black parts of the curves represent night measurements. The total driving force  $DF_{ABS}$  refers to an equal chlorophyll absorption.  $DF_{ABS} = DF_{\phi_{P_0}} + DF_{\Psi_0} + DF_{RC}$  where  $\phi_{P_0}$  refers to the primary photochemistry,  $\Psi_0$  to the dark reactions beyond  $Q_A^-$  and RC to the concentration of RCs.

SPECIFIC FLUXES PER RC    FLUX-RATIOS=YIELDS    FLUXES PER CROSS-SECTION



**Fig.3** : Pipeline models of energy fluxes measured on a polyp of an *Actinodiscus sp.* The top line corresponds to measurements taken at the 10<sup>th</sup> hour of the night, and the bottom line at the 10<sup>th</sup> hour of the day. Left: Membrane models showing the fluxes for absorption (ABS), trapping (TRo), electron transport beyond  $Q_A^-$  (ETo) and dissipation of PSII (DIo) all relative to one open reaction center (open circles). Middle: Flux ratios as quantum yields (per light absorbed ABS). Right: Phenomenological fluxes expressed per measured cross-section-area (CS) of the polyp. White circles represent intact open RCs, black circles represent  $Q_A$  non-reducing (heat sink) centers. Hatched areas in the membrane models (bottom left and middle) belong to the ABS, TRo and antenna of the heat sink centers. The width of each arrow indicates the maximal intensity of the fluxes absorption (ABS), trapping (TRo), electron transport (ETo) and dissipation (DIo). The oval symbolizes the average antenna size around one open intact reaction center.

## Results

Most parameters calculated from the fast fluorescence rise show clear circadian oscillations with a variable amplitude. In order to make the pattern of the oscillations clear we averaged several daily cycles (Fig. 1). The insert shows the mean of 11 selected days. However, averaging all days over the 6 months period gives a similar result.

An interesting point is that there is no correlation between the circadian rhythm and the moment at which the lights are respectively turned on and off. Even so, turning the lights on in the morning induces an increase in the rate of change of some of the parameters like  $\phi_{Po}$ ,

while on the other hand turning the lights off is not reflected. The inverse can also be true for some of the other parameters like trapping per reaction centers.

That the circadian rhythms are relatively independent of the ambient light was demonstrated by keeping a colony of *Porites porites* in darkness for 4 days (Fig. 2). The corals were able to maintain their circadian rhythm for a period of 3 days in the absence of light. The parameter plotted in Fig. 2 is also called driving force (DF) which is obtained by taking the log of the photosynthetic Performance Index ( $PI_{ABS}=10*RC/ABS*(\phi_{P_0}/1-\phi_{P_0})*(\Psi_0/1-\Psi_0)$ ).

The data from the long term experiment, collected this year between January 8 and June 11, were averaged. The induction curves measured at 6:00 h (2 h before lights on) and at 18:00 h (2 h before lights off) were selected and pipeline models (Strasser *et al.* 2000) for these time points were made. The pipeline models indicate that at the end of the day the PSII reaction centers trap energy more efficiently than at the end of the night, and at the same time that the number of reaction centers is low at the end of the day.

## Discussion

In the results section it was observed that photosynthesis in corals follows a circadian rhythm that is modified by external stimuli like light. It was also observed that in *Porites porites* this circadian rhythm can be maintained in darkness for at least 3 days. On the other hand the preliminary data presented here raise more questions than they provide answers.

For example does the observation that the  $\phi_{P_0}$  in these corals is lower during the night than during the day (Figs. 1 and 2) indicates that chlororespiration plays an important role in these organisms?

And which processes underlie the observed circadian rhythms? It is for example known that the expression of LHCII-genes depends on a circadian rhythm (Jacobshagen *et al.* 1996).

And finally, is the accumulation of photosynthetic products during the day leading to an inhibition of the photosynthetic chain?

We hope that further experiments will shed more light on these questions making our setup into a model system for the study of the effects of biotic and abiotic stresses on corals.

## Acknowledgements

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## References

- Strasser RJ, Srivastava A, Tsimilli-Michael M (2000) The Fluorescence Transient as a Tool to Characterize and Screen Photosynthetic Samples. In: Probing Photosynthesis: Mechanism, Regulation and Adaptation (Mohanty P, Yunus M and Pathre U, eds.), London and New York, Taylor & Francis, chapter 25, pp445-483.
- Tsimilli-Michael M, Strasser RJ (2001) Fingerprints for climate changes on the behaviour of the photosynthetic apparatus, monitored by the JIP-test. A case study on light and heat stress adaptation of the symbionts of temperate and coral reef foraminifers *in hospite*. In Fingerprints for climate changes (Wather GR, Burga C, eds) (2001) in press.
- Jacobshagen S, Kindle KL and Jonsohn CH (1996) *Plant Molecular Biology* **31**, 1173-1184.