Processes Affecting the Variability of Fluorescence Signals from Benthic Targets in Shallow Waters

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LONG-TERM GOAL

Our major theme is to understand and to qualify processes that contribute to fluorescence emission from benthic targets in the coastal and shallow waters with the overarching goal of developing parametrization schemes that optically detect anthropogenic objects.

OBJECTIVES

In the final CoBOP year we focused on analyzing data and developing models and algorithms that retrieve specific benthic targets based on fluorescence signatures and relate the variability in fluorescence to environmental conditions. These efforts are directed at developing optical closure.

APPROACH

Our basic method is the Fast Repetition Rate (FRR) Fluorometry which provides an extensive suite of fluorescence yields and photosynthetic parameters (Kolber et al, 1998). Two diver-operated FRR fluorometers have been developed for CoBOP and used for *in situ* and laboratory measurements in benthic targets, while the third, moored, instrument – for the study of temporal variability of fluorescence yields in selected organisms (Gorbunov et al. 2000; 2001). We also employed a number of standard laboratory techniques, including spectrofluorometry, spectrophotometry and biochemical isolations and characterization of particular chromophores.

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14. ABSTRACT
Our major theme is to understand and to qualify processes that contribute to fluorescence emission from benthic targets in the coastal and shallow waters with the overarching goal of developing parametrization schemes that optically detect anthropogenic objects.

15. SUBJECT TERMS

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WORK COMPLETED

In May 2001, we conducted final field experiments at Lee Stocking Island. Using the diver-operated FRR fluorometer, we acquired over five hundred measurements on corals, macroalgae and seagrass. The targets have been selected with the aim to complete the data set collected during the previous CoBOP years. Overall, about four thousand FRR measurements have been made and analyzed in four major functional groups of benthic targets (corals, macroalgae, seagrass, and sediments). Using the moored FRR instrument, we completed recording diel cycles of fluorescence signals in selected corals, macroalgae and seagrass. This data set has provided parameterization for the biophysical model describing temporal variability in benthic organisms.

RESULTS

FRR-derived fluorescence signatures of benthic targets - The FRR fluorometry allowed for specific optical signatures of benthic targets to be identified and quantified. The following parameters provide a basis for the algorithms that retrieve benthic targets from their fluorescent properties: chlorophyll fluorescence yields at minimum (F_o, open reaction centers) and maximum (F_m, closed reaction centers) levels; the quantum efficiency of light utilization in PSII (F_v/F_m); and the functional absorption cross sections of PSII (σ_{PSII}). While data analysis, benthic targets were divided into four major functional groups: corals, macroalgae, seagrass, and algal turfs on sediments. To avoid modulation of fluorescent data by irradiance induced fluorescence quenching (see below), we primarily analyzed measurements in a dark or low light adapted state.

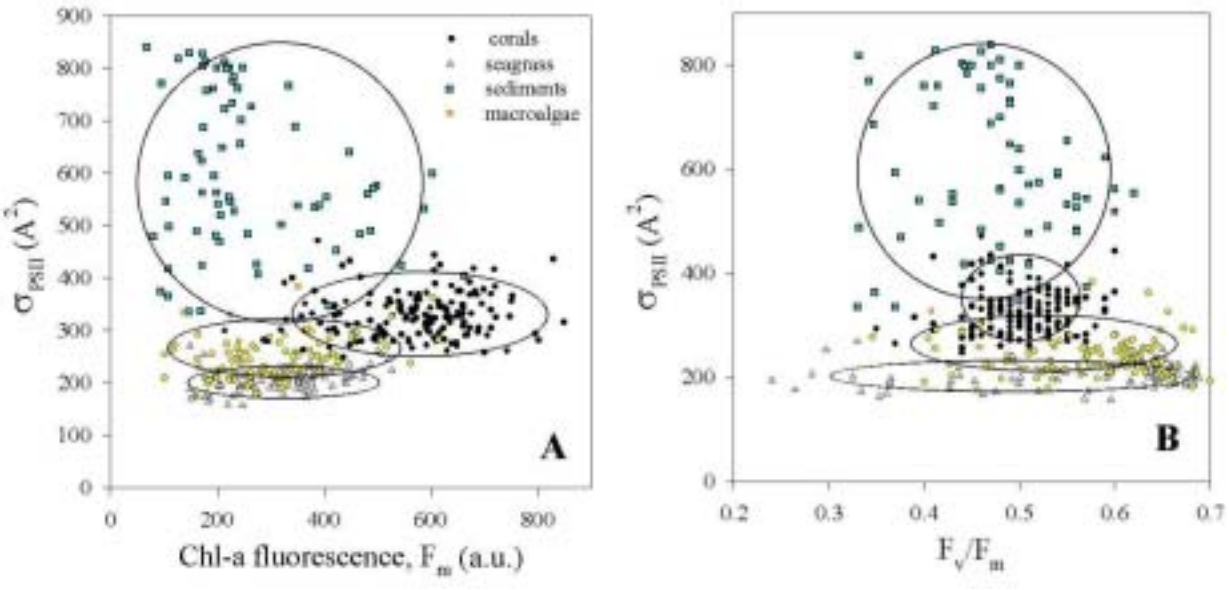

Figure 1. Fluorescent and photosynthetic signatures of major functional groups of benthic targets. (A) The functional absorption cross sections of PSII (σ_{PSII}) plotted as a function of chlorophyll fluorescence yield; (B) σ_{PSII} as a function of the quantum efficiency of light utilization in PSII (F_v/F_m). Note four clusters in both plots representing different types of benthic organisms.

Main results from this analysis are:

1. Fluorescence yields are highly variable within each functional group and overlap between the groups; the fluorescence yields along do not allow one to distinguish between different targets (Fig.1A).

2. Photosynthetic parameters (F_v/F_m and σ_{PSII}) are much more conserved and specific for each group.

3. Different benthic organisms exhibit unique sets of FRR-derived fluorescence and photosynthetic signatures that can be employed for identification of benthic targets.

4. Corals exhibit highly conserved photosynthetic efficiency (F_v/F_m averages 0.48) and functional absorption cross sections. Fluorescence yields, however, vary by a factor of ~3. This variability is primarily determined by variations in re-absorption of emitted fluorescence, while the molecular quantum yields are conserved.

5. Algal turfs have the highest σ_{PSII} and high photosynthetic efficiency (F_v/F_m averages 0.50). Fluorescence yields vary by an order of magnitude, depending on algal density.

6. Seagrasses exhibit the lowest σ_{PSII} and very high photosynthetic efficiency (F_v/F_m = 0.65 to 0.70), except of senescent and dead leaves (F_v/F_m = 0.25 to 0.4).

7. Macroalgae are characterized by the highest level of variability in both fluorescence yields and photosynthetic efficiency, attributed to environmental variations in the nutrient status of these benthic organisms.

8. The cluster analysis has revealed that the four major groups of benthic communities are reliably identified based on FRR fluorescent signatures.

Modeling diel variability in fluorescence yields – Since diel variability in fluorescence yields imposes a major problem for the interpretation and inter-calibration of fluorescent data collected at different time of the day and night, we specifically focused on the processes contributing to this phenomenon. Based on the comprehensive study of the mechanisms controlling the diel variability of fluorescence yields in benthic organisms (Gorbunov et al. 2001), we developed a biophysical model that allows to predict the diel patterns of chlorophyll fluorescence from the knowledge of irradiance and photosynthetic parameters. The model has been tested and parameterized against an extensive data set (about 150 diel cycles) acquired during the 3rd to 5th CoBOP years.

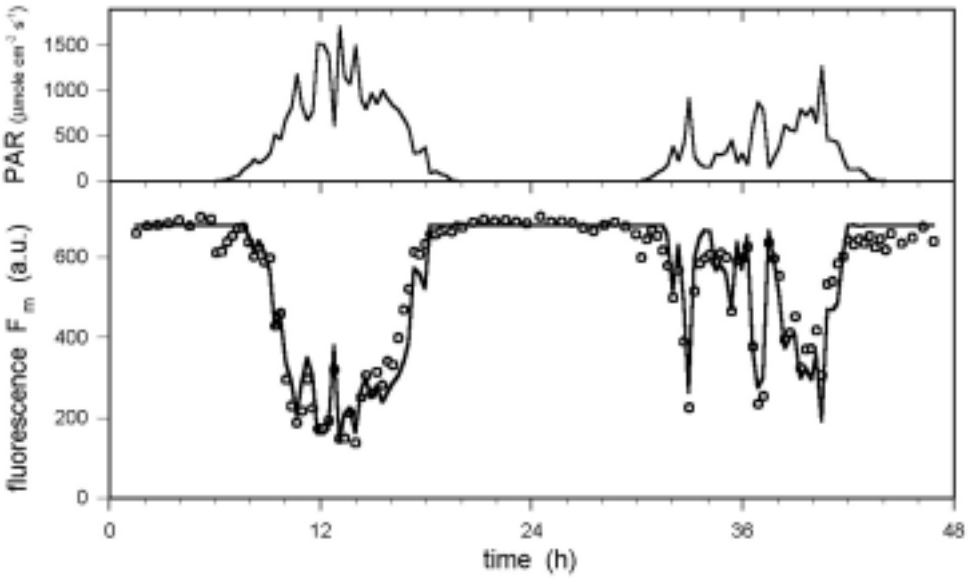

Figure 2. Diel cycles of chlorophyll fluorescence yield at the maximum level (F_m) recorded in the coral M.faveolata in shallow water (2m depth). Upper panel – diurnal variations in PAR irradiance. Lower panel – measured values (points) together with the predicted pattern (line). [Fluorescence yield decreases 3 to 4 times during a day under high irradiance. Deviations of the measured values from the predicted line are about 10% of F_m]

The model relies on the indirect regulation of fluorescence yields by irradiant variations in photosynthetic activity. In photosynthesis, the energy of absorbed light may be used for photochemical conversion, dissipated to heat (non-photochemical quenching) or re-emitted as fluorescence. The quantum efficiencies of the three pathways sum to unity, but each is strongly controlled by ambient irradiance. A by-product of photosynthetic processes, fluorescence exhibits low quantum yields (~2 to 6%) and can be modeled from the knowledge of the two other pathways - photosynthetic conversion and thermal dissipation. The rates of photochemical conversion follow the photosynthesis-versus-irradiance curve, P(E), which is retrieved from the FRR fluorescence measurements (Gorbunov et al., 2001). The rates of thermal dissipation are directly driven by the extent to which the photosynthetic electron transport is saturated at any given irradiance (Gorbunov et al. 2001). Therefore, the irradiance dependence of the quantum efficiencies of non-photochemical quenching is assessed from the irradiance dependence of the photochemical quantum efficiency, $\Delta F'/F_m'$. Then, the knowledge of non-photochemical quenching allows the diurnal patterns of fluorescence yields (at both steady state F', and maximum, F_m', levels) to be calculated. Fig. 2 shows an example of the measured and predicted diel cycles of F_m' (this fluorescence yield corresponds to fully closed reaction centers of Photosystem II and is recorded when saturating excitation light is employed, e.g., by the Laser Line Scanner). Comparison of the modeled and measured profiles (150 diel cycles) revealed that the model accounts for ~80% of the variability in fluorescence yields. The analysis suggests that the minor errors in the modeling diel variability are determined by two factors. First, the circadian rhythm of photosynthetic activity causes "hysteresis" in the irradiance dependence of photosynthesis, leading to under-estimation of fluorescence quenching in the morning and its over-estimation in the evening. The extent of this hysteresis may vary between species and even within a common species. Second, at a given instant, fluorescence yield is determined not only by the instant

irradiance, but also "pre-illumination history". Following the induction and relaxation of photosynthetic activity, fluorescence yield exhibits a delayed response to a change in irradiance. The fast photoadaptive responses in corals and a number of macroalgae appear to have minor impact on the measured diel cycles in these organisms, but this effect is significant in slowly responding organisms, such as seagrass and green algae, when irradiance varies rapidly under cloudy skies. Further incorporation of relaxation/induction parameters into the model, in conjunction with high-resolution records of irradiance, permits the precision of modeled profiles to be improved.

Effect of polyp expansion/retraction on optical properties of corals and the mechanisms controlling this behavior – A large number of benthic creatures are only active at night. Light-driven expansion/retraction rhythms are typical for many of these organisms, including corals, gorgonians, and sea anemones (for example, see images of the coral *M. cavernosa* made under ambient light and in darkness in Fig. 3A). As a consequence, the color appearance of coral reefs differs significantly between day and night. Expanded polyps increase scattering of incident light by coral surface, thus affecting spectral reflectance. Furthermore, we found that in some species polyp expansion in darkness leads to a significant change in the green and/or orange fluorescence emission (Fig. 3A). Since this fluorescence emission may contribute up to ~ 50% to the reflected light at the wavelengths of maximum emission (Mazel, 1997), this effect must be considered while comparing and interpreting measurements of optical properties made at different times of day and night. We studied the mechanisms behind this phenomenon on selected model coral species (Gorbunov and Falkowski 2001). We measured the photoreceptive responses by monitoring the effect of irradiance on the contraction of polyps in coral species that normally have extended tentacles in darkness. The response was recorded using a laser-based scatterometer, developed for this study (Gorbunov and Falkowski 2001). Main results of these studies are:

1. Expansion/retraction of polyps is primarily driven by the level of ambient light.

2. This behavior is regulated by sensitive photoreceptors located in the host animal of coral symbioses

3. The detected threshold of photoreception sensitivity is as low as ~1.2 * 10^{15} quanta m^{-2} s^{-1} in the blue spectral region. As a consequence, major changes in the expansion/retraction of corals occur under low irradiance (at sunrise and sunset).

4. The extraordinary sensitivity of coral photoreceptors makes them capable of sensing the blue portion of lunar irradiance, that is supported by minute variations in polyp extension recorded under variations in the moonlight intensity.

5. The action spectra of coral photoreception reveal a maximum sensitivity at 480 nm, with a spectral half band width of ca. 110 nm (Fig. 3B). The spectra closely overlap the maximal transparency of oligotrophic tropical waters, thus optimizing the perception of low light at depth;

6. The action spectra of photoreception in corals (Fig. 3B) are remarkably similar to the absorption spectra of rhodopsins isolated from a number of marine invertebrates, that rises the possibility that corals may have rhodopsin-like photoreceptors.

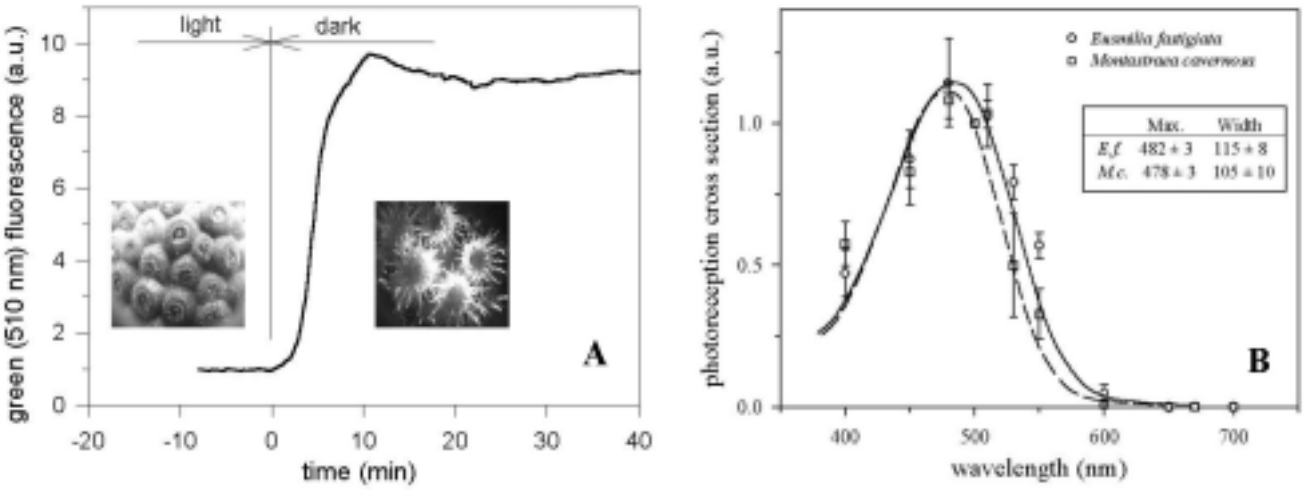

Figure 3. (A) Effect of polyp expansion on the intensity of green fluorescence (emission at 510 nm) from the coral Montastraea cavernosa. The images in the insert show the coral with retracted and extended tentacles - under ambient light and in darkness, respectively.
[Green fluorescence increases 10 times in darkness when polyps become extended]

Figure3. (B) Action spectra of photoreception in the corals E. fastigiata and M. cavernosa. The blue sensitive photoreceptors have been found to control the state of polyp retraction, thus modifying optical properties of these organisms. The calculated peaks of the spectral sensitivity and the spectral half band widths (both in nanometers) for the two corals are shown in the insert.
[The spectra have maximum at 480 nm and a half band width 110 nm]

IMPACT/APPLICATIONS

Understanding the sources of variability and behavior of benthic fluorescent targets is essential to developing operational protocols for distinguishing between anthropogenic and naturally occurring objects. Moreover, within the overall goals of CoBOP, namely identification and quantitation of IOPs that are required for closure of radiative transfer models, fluorescence is a source of spectrally camouflaged photons that are radiated from benthic targets that have absorbed photons at other wavelengths. Thus, the consideration of varying fluorescence processes, in conjunction with the measurements of spectral absorption and scattering, will lead to numerically accurate and complete radiative transfer models. Understanding the patterns and mechanisms of diel variability in fluorescence yields is needed for the interpretation of optical data collected at different times of day and night. Specifically, this allows the cross-calibration of the night data by the Fluorescent Laser Line Scanner and the day time measurements by supporting fluorescent techniques.

TRANSITIONS

Dr. Michael Lesser (Univ. of New Hampshire) used the SCUBA-based FRR fluorometers in an ONR-funded project, on the effect of spectral quality and quantity in the underwater light field and elevated temperatures on small-scale optical properties of corals. As part of collaborative CoBOP effort, Dr. Charlie Mazel uses FRR data in the interpretation of fluorescent signatures of corals. Dr. C.W. Wright (GFSC, NASA) uses our data and knowledge gained during the CoBOP for interpreting the results of

LIDAR remote sensing of coastal zones and coral reefs. Also, Professor Zvy Dubinsky and his colleagues at Bar-Ilan University (Ramat Gan, Israel) employ one of the FRR instruments for the study of coral reef optical properties in the Red Sea.

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