Technical discussion III

Fluorescence measurements*

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SUMMARY: The applications, problems and limitations of fluorescence measurements in photophysiology are discussed. Great care is required in interpreting fluorescence measurements from algae, and in particular in comparing results from different taxa. The existing pulse-amplitude modulated (PAM) instrumentation seems to be satisfactory as far as macroalgae are concerned, and the way is open for novel work on photosystem physiology, regulation and ecological adaptation.

Key words: Fluorescence, pulse-amplitude modulated (PAM) instrumentation, quantum yield, technical discussion.

This technical discussion covered fluorescence measurements from both phytoplankton and macroalgae. In spite of some close taxonomic relationships between the two groups they present very different measurement problems since the pigment concentrations seen in the sensing volume of a fluorometer differ by several orders of magnitude. Furthermore Wilhelm pointed out that algal photosystems varied greatly from one taxonomic group to another, and that only the green algae had pigment and chloroplast configurations similar to those that have been well studied in higher plants. Consequently great care is required in interpreting fluorescence measurements from algae, and in particular in comparing results from different taxa. He drew specific attention to the following points concerning the interpretation of signals from a Schreiber-type pulse amplitude modulated (PAM) system:

Measurement of $F_o$

$F_o$ is defined as the fluorescence emission from the sample when all the reaction centres are open, or $Q_A$ is fully oxidised. The PAM measuring light is asserted to be 'non-actinic', but in fact the wavelength is well absorbed and the energy of each quantum is sufficient to potentially drive charge separation and to close a reaction centre. Only under conditions where the rate of closure is less than the rate of re-opening does the population of open state reaction centres accumulate, and the light flux required to close a significant number of the reaction centres depends on the size and efficiency of the light harvesting antenna of PSII. Many algae possess much larger antennae than those found in the chloroplasts from higher plants, and consequently lower measu-

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ring light intensities must be used to keep the reaction centres open. However the use of such low measuring light intensities can reduce the fluorescence signal to the point where \( F_v \) measurements become too noisy to be useful.

**Low \( F_v/F_m \) ratios**

In green algae and higher plants \( F_v/F_m \) is generally around 0.8, but in some algae this ratio can be as low as 0.4 to 0.7 (Büchel and Wilhelm 1993). Similar low values have been found in natural phytoplankton samples, where their occurrence is usually interpreted as an indicator of photooxidative or nutrient limitation. However there is good evidence that \( F_v/F_m \) differs significantly between algal classes due to variations in the supramolecular organisation of the thylakoid membrane system. Contributory factors include:

a. An increase in \( F_m \) due to a high proportion of uncoupled antennae molecules. This seems to be the case in some Prasinophytes like *Mantoniiella* (Trissl and Wilhelm, unpublished observations).

b. A decrease in \( F_m \) due to very efficient spillover from PSII to PSI. Many chlorophyll c containing algae do not possess true grana in the sense that PSII and PSI are spatially separated, and this increases the spill-over rate significantly (Trissl and Wilhelm, 1993).

c. Very fast state 1/state 2 transitions in phycobilisome containing algae. In this case, one observes that \( F_m \) is nearly lost after the first light saturating pulse of about 500 ms. This pulse is sufficient for an uncoupling of the phycobilisomes from PSII and an α-transfer of energy to PSI, which is an efficient fluorescence quencher.

d. A decrease in \( F_m \) due to ‘energy dependent’ quenching even after dark adaptation. In some algal species there is electron flow via NADPH-PQ-oxidoreductase in the dark. This electron flow generates a pH gradient which has effects on \( F_m \) similar to energisation of the thylakoid membrane in the dark-light transition (Wilhelm and Duval, 1990). The existence of a pH gradient in the dark can be checked by the addition of an uncoupler just before the second \( F_m \) measurement. Dubinsky added a further note of caution by pointing out that the term photooxidation was often mis-used in field studies when adaptive or photoprotective mechanisms are involved.

Levavasseur, while accepting the need for careful signal interpretation, reported satisfactory results obtained using a Hansatech instrument on seaweeds in the laboratory. Hanelt described practical aspects of the use of a Walz portable PAM for seaweed measurements in the field, paying particular attention to the requirement for a computer which consumed little power and had a screen which was readable in daylight. The portable instrument made field work possible in remote locations, even though the actinic light source used was less flexible than that which could be deployed in the laboratory.

Estrada emphasised that biological oceanographers were under considerable pressure to produce measurements on phytoplankton abundance at similar rates to those achieved for physical variables such at temperature and conductivity. Optical measurements, primarily fluorescence, offered the only hope of this rate of data acquisition even though the interpretation of *in-situ* oceanographic fluorometry was extremely difficult. It was noted that the only submersible fluorometers that were currently readily available were single-flash devices, so that measurement options in general oceanography were severely limited. A recent development of the PAM system (Schreiber et al., 1993) had greatly increased its sensitivity, but no description of a submersible version had been published. There were plans for a commercial version of the Brookhaven FRR fluorometer (EMS, 1993) which would be of great interest.

Overall, existing pulse-amplitude modulated (PAM) instrumentation seems to be satisfactory as far as macroalgae are concerned, and the way is open for novel work on photosystem physiology, regulation and ecological adaptation. PAM equipment has been successfully used for measurements on relatively dense phytoplankton cultures, but natural populations generally occur at concentrations which are too low for measurements using commercial PAM equipment. For phytoplankton, the lack of submersible instrumentation suitable for photobiological studies remains a major obstacle to progress.

**REFERENCES.**


