Study on chlorophyll fluorescence responses of phytoplankton under nutrient-limited growth conditions

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Introduction

Nutrients in inner bay areas are supplied from multiple sources, including rivers, groundwater, and diffusion from sediments, each characterized by distinct fluxes, nutrient ratios, and spatiotemporal distributions. While nutrient limitation on phytoplankton growth is generally assessed through incubation experiments with nutrient addition, these methods pose challenges: they require several days to complete and are difficult to implement for spatial mapping due to the complexity of the experimental procedures and the resulting constraints on the number of sampling stations. The objective of this study was to develop a method for the simple, on-site evaluation of nutrient-limited photosynthetic activity and the identification of growth-limiting nutrient species. To achieve this, we measured changes in photosynthetic parameters in nutrient-limited phytoplankton calculated from chlorophyll fluorescence and conducted experiments on Nutrient-Induced Fluorescence Transient (NIFT)-a phenomenon where the re-supply of a deficient nutrient causes short-term changes in chlorophyll fluorescence.

Materials and Methods

Three isolated culture strains that represent typical phytoplankton in Tokyo Bay were used: the diatoms *Thalassiosira allenii* and *Skeletonema ardens*, and the dinoflagellate *Prorocentrum triestinum*. First, these strains were subcultured into SWM-III media deficient in nitrogen (N), phosphorus (P), or silica (Si). Next, photosynthetic parameters were measured using a Water-PAM-II to detect nutrient-limited states and compare the responsiveness to nutrient deficiency of each species. The evaluation was primarily based on changes in Fv/Fm, supplemented by light curves generated for ETR, Y[II], qP, NPQ, and Fv'/Fm'. Furthermore, in the NIFT experiments, the deficient nutrients were re-supplied to samples identified as nutrient-limited. After establishing a fluorescence baseline, nutrients were added in two stages (low and high concentrations), and the resulting changes in fluorescence were recorded.

Results and Discussion

As a result of the nutrient limitation treatments, Fv/Fm, ETR, Y[II], and Fv'/Fm' showed a downward trend compared to the control in all experimental groups. For example, the decrease in Y[II] and ETR in the P-deficient group of *T. allenii* is thought to be related to a reduction in electron demand caused by decreased ATP synthesis and RuBisCO activity due to P depletion. Conversely, interspecific differences were observed in the responses of qP and NPQ. Notably, a decrease in qP did not occur only under Si-limited conditions in the diatom species. Among the overall decline in Fv/Fm and Y[II], specific characteristics were observed in the N-limited group of *P. triestinum*, where values recovered for several days after an initial drop and then declined again.

In the NIFT experiments, no distinct fluorescence response was observed upon the addition of N or P for any species of the phytoplankton. However, when the slopes of the fluorescence graphs were examined specifically at three measurement points (approximately one minute) before and after addition and compared with the control experiments, some samples showed changes in slope following addition. These may indicate physiological responses to changes in nutrient concentrations.

Keywords: Phytoplankton, Chlorophyll Fluorescence, Pulse Amplitude Modulation, Nutrient

