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Delayed luminescence of *Prorocentrum minimum* under controlled conditions

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Abstract

Delayed luminescence (DL), also termed delayed fluorescence or delayed light emission, is the phenomenon of long-lived light emission by plants and cyanobacteria after being illuminated with light and put into darkness. Culture growth of three *Prorocentrum minimum* strains was studied with DL measurements. DL decay kinetics was measured from 1–60 s after a pulse of white light. The strains used were from the Adriatic Sea (PmK), from Chesapeake Bay, USA (D5), and from the Baltic Sea (BAL), cultured at salinity of 32, 16, and 8 (practical salinity scale), respectively. The strains differed in cell size and chlorophyll *a* content (PmK > D5 > BAL), as well as in DL parameters. The DL results were compared to standard measurements of culture density and carbon content (calculated from biovolumes). DL decay curves had a specific peak, which changed with culture growth and showed more similarities between the strains PmK and D5. The DL intensity increased with cell density and carbon content in a two-stage process, corresponding to the lag and exponential phases of growth. DL intensity was best correlated with carbon content irrespective of strain and is proposed as an estimate of biomass and for differentiating between lag and exponential phases of growth.

Keywords: *Prorocentrum minimum*; Delayed luminescence; Growth phases

1. Introduction

Prorocentrum minimum (Pavillard) Schiller is a common bloom-forming dinoflagellate in temperate waters and is potentially toxic (Okaichi and Imatomi, 1979; Shimizu, 1987; Grzebyk et al., 1997; Denardou-Queneherve et al., 1999). It has a wide geographical distribution and its blooms generally occur in zones affected by freshwater and/or anthropogenic inputs (Grzebyk and Berland, 1996; Witek and Plinski, 2000). The ability of *P. minimum* to survive and grow under nutrient- and light-stressed conditions has been

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studied in several field (Tyler and Seliger, 1981; Harding, 1988; Harding and Coats, 1988; Stoecker et al., 1997) and laboratory investigations (Faust et al., 1982; Coats and Harding, 1988; Sciandra, 1991; Grzebyk and Berland, 1996).

Growth of *P. minimum* shows no strict requirements for temperature, light, or salinity conditions (Grzebyk and Berland, 1996). It grows in a temperature range between 8 and 31 °C, with an optimum between 18 and 26.5 °C. Variations in light intensity between 30 and 500 μmol photons m^{-2} s^{-1} has little effect on its growth. The optimal salinity range is from 15 to 35 PSU, and, after adaptation, slow growth occurs at salinities as low as 5. Higher growth rates can be observed at intermediate salinities (15–17 PSU; Hajdu et al., 2000).

Delayed luminescence (DL), also termed delayed fluorescence or delayed light emission, is the phenomenon of long-lived light emission by plants and cyanobacteria after being illuminated with light and placed in darkness (Strehler and Arnold, 1951). In contrast to rapid fluorescence, which is light emission on the nanoseconds time-scale, the characteristic times for DL are from milliseconds to seconds. The emission spectrum resembles the fluorescence emission spectrum of chlorophyll *a* (Arnold and Davidson, 1954; Van Wijk et al., 1999). DL originates from the repopulation of excited states of chlorophyll from stored energy after charge separation (Jursinic, 1986), whereas rapid fluorescence reflects the radiative de-excitation of excited chlorophyll molecules before charge separation.

The main source of DL is photosystem II (PSII) in the thylakoid membrane of chloroplasts. Charge pairs are generated in PSII during illumination, with positive charges located on the oxygen-evolving complex (OEC) and negative charges on quinone acceptors (Q_A and Q_B). The slow components of DL originate in back reactions between the S_2 and S_3 states of the OEC and quinones Q_A and Q_B (Joliot et al., 1971). The half-times of these reactions in isolated chloroplasts are 1.5 s for $Q_A + S_{2/3}$ and 25 s for $Q_B + S_{2/3}$ (Rutherford and Inoue, 1984). DL has hyperbolic decay kinetics in the first seconds (Scordino et al., 1996), sometimes followed by a more or less pronounced peak (Milani et al., 2003). Desai et al. (1983) observed the peak after illuminating the sample with far red light. One or more transient peaks were induced also by phosphorus starvation and carbon dioxide deficiency (Mellvig and Tillberg, 1986).

Delayed luminescence intensity (DLI) represents the integral under the decay curve and is an increasing function of the number of PSII centers, the fluorescence yield, and the rate of back reactions, which are influenced by the membrane potential and pH gradient (Joliot et al., 1971; Wraight and Crofts, 1971; Avron and Schreiber, 1979; Joliot and Joliot, 1980). In field studies, DLI was reported to be a measure of photosynthetic activity (Schneckenburger and Schmidt, 1996). Krause and Gerhardt (1984) and Wiltshire et al. (1998) have shown that DL can be applied in limnology and oceanography and that DLI of phytoplankton is a measure of living algal biomass. Yacobi et al. (1998) have analyzed natural phytoplankton samples using DL excitation spectra and were able to detect taxonomical changes in the algal communities. This technique is now used in monitoring freshwater phytoplankton (Hakanson et al., 2003). To our best knowledge, no studies have yet been published on *P. minimum* DL kinetics and DLI variation during different growth phases.

The aim of the present study was to monitor the changes in DLI of *P. minimum* during culture growth and to qualitatively compare DL decay curves of three *P. minimum* strains at different growth phases. The dynamics of DLI was followed during successive growth phases under controlled conditions and expressed in dependence on culture density and carbon content (calculated from cell volumes). DL decay kinetics was measured after white light illumination over 1–60 s.

2. Materials and methods

2.1. Algal cultures

The Baltic strain of *P. minimum* (BAL) was collected in the Baltic Sea and isolated at the Kristineberg Marine Research Station, Sweden. The American *P. minimum* (D5) was obtained from D. Stoecker, originally isolated from Chesapeake Bay, USA. The Adriatic *P. minimum* (PmK) was isolated from the Gulf of Trieste, Italy.

The stock cultures were maintained at the Laboratory of Marine Biology, Trieste, at 15 °C,