14

March, 1992 Volume 5, No.1

Signal & Noise
Individual Particle Analysis in Aquatic Sciences

Phototrophic Pico- and Nanoplankton in the Central Baltic Sea: Estimates by Fluorescence Microscopy and Flow Cytometry

Andrea E. Detmer & Frank J. Jochem
Institut für Meereskunde, Dustenbrooker Weg 20, 2300 Kiel, Germany

Our previous studies in the Baltic Sea have shown that phototrophic picoplankton contribute a major part of chlorophyll standing stocks and primary productivity during summer. Thereby, phototrophic organisms <5 µm are dominated by *Synechococcus*-type cyanobacteria (Jochem, 1988). Studying dynamic processes of the microbial food web in the Central Baltic Sea (57° 22'N, 19°55'E) during cruise BAMBI II (Baltic Microbial Biology Investigation) in July/August 1991, vertical profiles of abundance of *Synechococcus* and phototrophic flagellates <5 µm were obtained by both fluorescence microscopy and flow cytometry. Besides an evaluation of pico- and nanoplankton distribution in relation to environmental conditions, the cell numbers obtained by both techniques were compared.

Temperature profiles were recorded by a ME-CTD probe. Water samples were taken with black Niskin-type water bottles each day of the two 5-day drogue stations in the morning (0630 to 0800). Oxygen concentrations were estimated by Winkler titration and the H_2S concentrations after Grasshoff et al. (1983). For fluorescence microscopy, 20-40 ml of fresh sample were filtered onto 0.2 µm Irgalan Black pre-stained Nucleporefilters. Phototrophic organisms were counted under a Zeiss epifluorescence microscope using blue light excitation (450-490 nm) and a 100x Neofluar objective. *Synechococcus* and phototrophic flagellates could easily be distinguished by their different autofluorescence. 150 - 200 cells were counted for each organism group, yielding a mean error of 10-15%. Cytometric analysis was performed on a FLUVO II flow cytometer constructed by Dr. V. Kachel, Max-Planck-Institut Munich, using the same blue light excitation. Phycoerythrin was measured as 530-585 nm emission and chlorophyll as >615 nm emission.

The water column was characterized by a pronounced thermocline at about 10 m depth. Oxygen increased down to 50 m, sharply

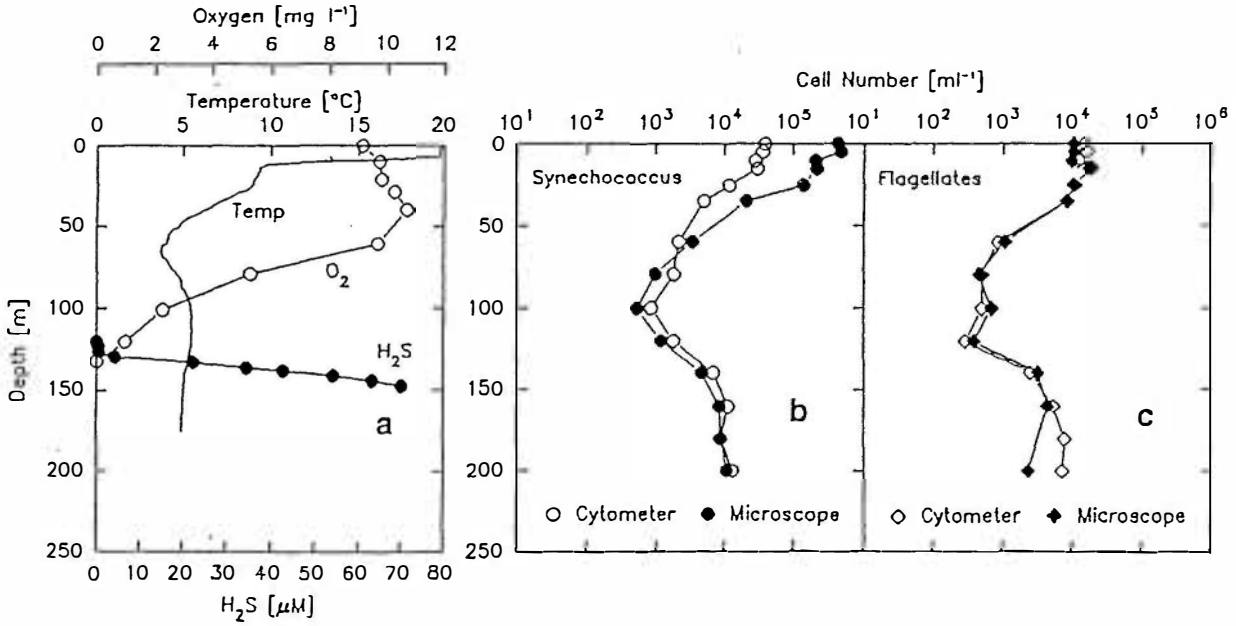

Fig. 1 a, : Vertical profiles of temperature (°C), oxygen (mg l^{-1}) and H_2S (µM) at a drogue station in the Central Baltic Sea. Vertical profiles of cell numbers (l^{-1}) (b) of Synechococcus and (c) phototrophic flagellates <5 µm estimated by fluorescence microscopy and flow cytometry at a drogue station in the Central Baltic Sea

decreasing below. At 120 m depth, the permanent chemocline - characteristic for the Central Baltic - was encountered. Oxygen concentrations having dropped to zero and H_2S increased to about 70 µM within a few meters (Fig. 1). Both *Synechococcus* and flagellates displayed high abundances near the surface, decreasing below the euphotic zone (25 m) with lowest cell numbers in the mid-water layers (Figs. 1 b, c). Related to the chemocline, an increase of cell numbers was obvious again. Similar profiles and cell numbers were obtained throughout the 10 day study period. Within anoxic waters, the abundance of *Synechococcus* amounted to 1 - 2% of surface values and to 50% for flagellates. Whereas *Synechococcus* were 100 times more abundant than flagellates in the upper 50 m, cell numbers of both were similar at greater depths. Phototrophic organisms of anoxic waters were brightly fluorescent and alive, still capable of primary production after re-oxygenation of the water and under light (Detmer et al. in prep.).

The comparison of microscopy and flow cytometry shows fairly comparable cell numbers for flagellates (Fig. 2), the slope of the regression line being close to 1. Although the regression for *Synechococcus* also is highly significant ($p<0.001$) the slope is far from a 1:1 relation (Fig. 2). Vertical profiles (Fig. 1b) show that this is basically due to a high discrepancy in the upper 50 m. Here, *Synechococcus* tended to aggregate which led to underestimation by flow cytometry. Hitherto, only a few similar comparisons are published. Pronounced discrepancies near the surface were also found by Olson et al. (1985) and Burkill & Jochem (unpubl. data) although Atlantic *Synechococcus* normally do not form aggregates. Further comparisons of both techniques in field studies

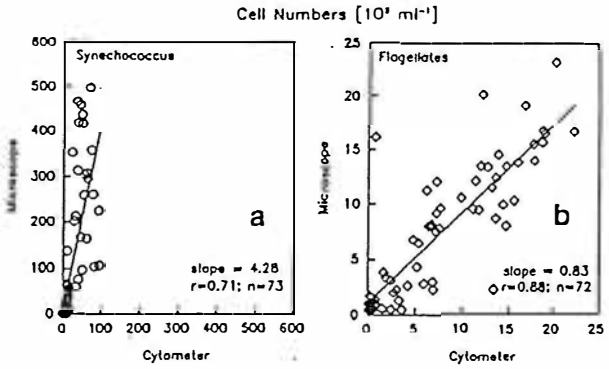

Fig. 2 Correlation of cell numbers obtained by fluorescence microscopy and flow cytometry for (a) Synechococcus and (b) phototrophic flagellates <5 µm of all field samples of cruise BAMBI II.

therefore are highly recommended.

References

Detmer, A.E., V.M. Trenkel, H.C. Giesenhagen, H. Auf dem Venne, F.J. Jochem (in prep.) Phototrophic and heterotrophic pico- and nanoplankton in anoxic depths of the Central Baltic Sea.

Grasshoff, K., M. Ehrhardt, K. Kremling (1983) Methods of Seawater Analysis, 2nd ed. Verlag Chemie, Weinheim. 419 pp.

Jochem, F.J. (1988) On the distribution and importance of picocyanobacteria in a boreal inshore area (Kiel Bight, Western Baltic). J. Plankton Res. 10. 1009-1022.

Olson, R.J. , D. Vaulot, S.W. Chisholm (1985) Marine phytoplankton distributions measured using shipboard flow cytometry. Deep-Sea Res. 32. 1273-1280.