bbe
MOLDAENKE

Schauenburgerstr.116
D - 24118 Kiel
Telefon 0431/5 606 606
Telefax 0431/5 606 609

e-mail BBE.Moldaenke@t-online.de
Internet http://home.t-online.de/home/BBE.moldaenke/

FluoroProbe

- a new tool in algal differentiation -

by Martin Beutler

Summary:

The 'spectral groups' of algae (green, blue, brown, mixed) are each characterised by a specific composition of photosynthetic pigments (particularly relevant are: Chl a, phycocyanobilin, phycoerythrobilin, fucoxanthin, peridinin) and, consequently, by a specific excitation spectrum of the Chl fluorescence. The newly-developed probe is a submersible fluorometer which measures the emission intensity for excitation in five characteristic wavelength ranges employing pulsed light-emitting diodes. The submersible probe transfers all data on-line to a personal computer or stores them in the probe (fluorescence data plus the simultaneously measured water pressure for depth determination). The five-point excitation spectra (5 wavelength ranges) are deconvoluted on the basis of norm spectra which have been obtained by analysis of several species of each spectral group. This enables an estimation of the mean deviation of the norm spectra. By means of the deconvolution approach, for each spectral algal group an estimate of the corresponding Chl a concentration is obtained (µg of Chl a L^{-1} per spectral algal group in the measuring volume at the given depth).

Introduction:

The differentiated assessment of the phytoplankton distribution is a prerequisite for a qualified estimate on the rate of primary production by phytoplankton and on its dependence on environmental factors. Also, supervision of the phytoplankton can facilitate the early identification of an unusual or stressed status of the aquatic ecosystem (e.g., algal blooms, toxic substances, oxygen deficit, etc.).
The presently available methods for determination of the phytoplankton distribution in waters often lack the spatial and temporal resolution required to obtain a thorough understanding of the role of phytoplankton in aquatic ecosystem. The typical delay between sampling a water volume and obtaining the final results mostly excludes the use of conventional methods for supervision or monitoring tasks. Furthermore, presently established methods are often highly costly in terms of manpower.

In oxygenic photosynthesis, the chlorophyll (Chl) fluorescence measured around 685 nm is predominantly emitted by the Chl of the photosystem II (PS II) antenna system which consists of the evolutionarily conserved Chl *a*-core antenna and

species-dependent peripheral antennae. According to the composition of the peripheral antennae spectrum there can be found five main spectral groups (Fig. 1). In the ‘Green Group’ the peripheral antennae consist of chlorophyll-a, -b and xanthophyll. The phycobilisomes of the ‘Blue Group’ function as peripheral antennas. These are rich in phycocyanin. The members of the ‘Brown Group’ contain Chlorophyll-a and -c and xanthophyll. This is often either fucoxanthin or peridinin. The peripheral antennae of the ‘Red Group’ are composed of phycobilisomes as in the ‘Blue Group’. But the phycobiliprotein phycoerythrin is dominating in the ’Red Group’ instead of the phycocyanin. However the ‘Red group’ plays an unimportant role among the planktic algae and is commonly distributed as benthic species. The ‘Mixed Group’ has a very special pigment composition. Here there is a combination of Chlorophyll-a, -c, with one phycobiliprotein which can be either phycoerythrin or phycocyanin. In this work just the phycoerythrin-containing members of the ‘Mixed Group’ are considered.

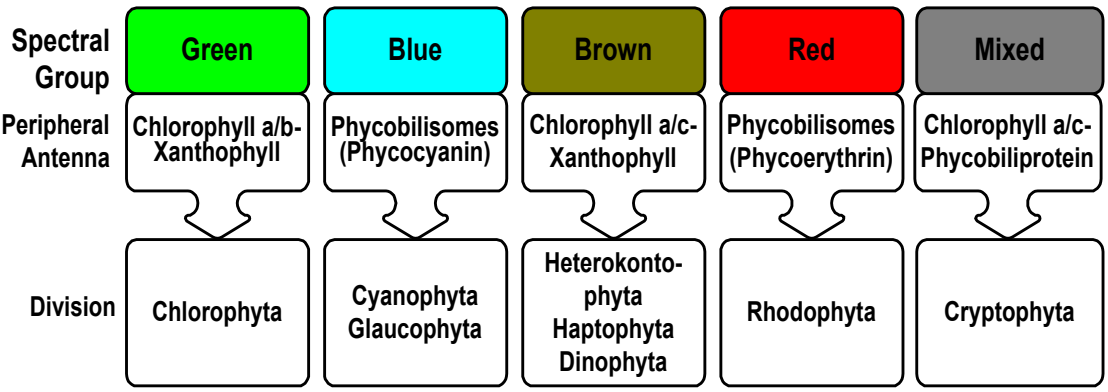


Fig. 1: Assignment of several algal divisions in spectral groups

We measured fluorescence excitation spectra for several species of each spectral group using five distinct excitation wavelengths. The mean excitation probabilities per Chl *a*-concentration (in instrument dependent units) are shown in Fig. 2. Using the 'norm-spectra' shown in Fig. 2, it becomes possible to determine quantitatively the algal population distribution (meaning the Chl *a*-concentration per spectral alga group) within the sample volume of a submersible instrument. It is worth mentioning that the Chl *a*-concentration per spectral algal group is a particularly useful parameter because it is closely related to the rate of primary production per water volume.

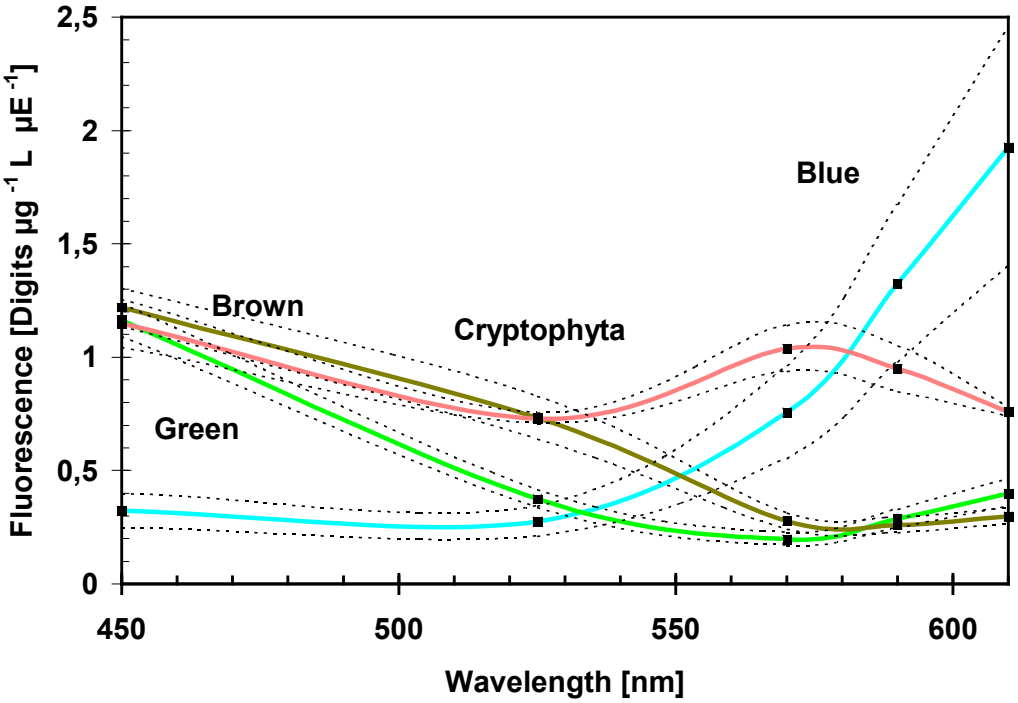
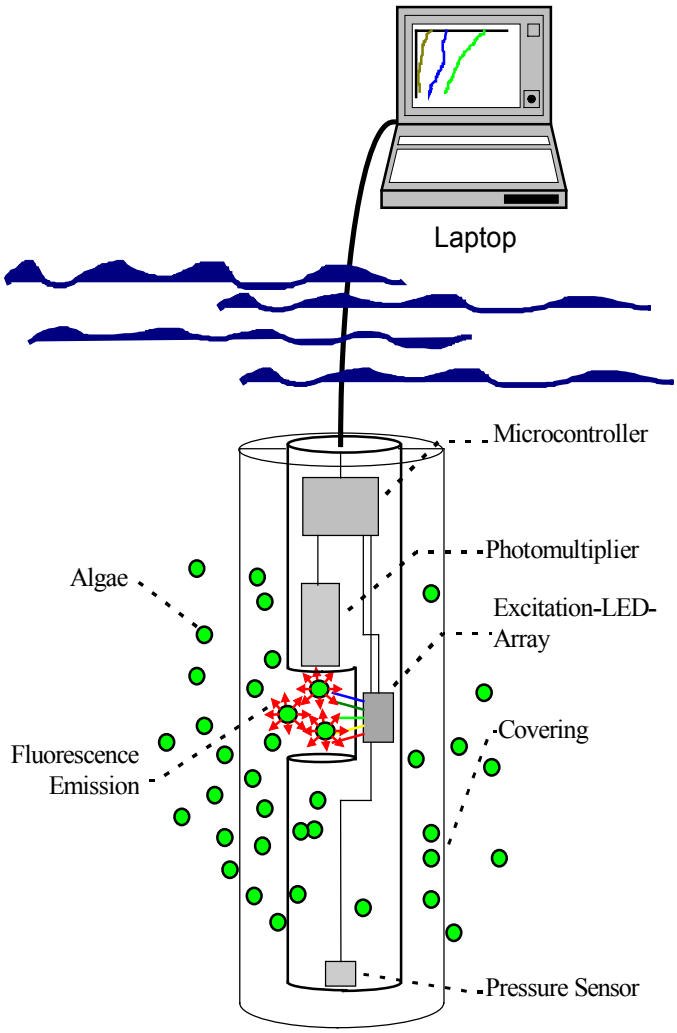


Fig. 2: Norm spectra of the spectral groups and their standard deviations:
Mean fluorescence intensity of the four spectral groups at five excitation wavelengths in Digits (photovoltage at the photomultiplier) normalized to chlorophyll-a concentration (μg^{-1} L) of the sample and the incident quantum number at the excitation-wavelength (μE^{-1}). The slashed lines give the standard deviation calculated for several species from one spectral group. There is a high standard deviation for the 'Blue Group' in the red wavelength region (610 nm). This is caused by the variability of the phycobilisomes. In addition the 'Brown Group' (here diatoms and dinoflagellates) has a high standard deviation in the green wavelength region (525 nm) because of the differences that occur with fucoxanthin- and peridinin-containing algae. High fluorescence intensity can be found within the cryptophyta (cryptomonas) at 570 nm caused by phycoerythrin.

This knowlegde and technique was integrated into the bbe FluoroProbe with the following characteristics: robust windows and stainless-steel housing (l = 45 cm, ∅ = 14 cm) suitable for water-depths up to 100 m; continuous monitoring of the submersion depth by means of an integrated pressure sensor; five-color excitation using long-living light-emitting diodes (LED); rectangular detection at the PS II-fluorescence emission peak (685 nm) using an optimized optical bandpass-filter combination and a robust, red-sensitive miniature photomultiplier; microprocessor-control of LED-pulse sequences and data acquisition; high sensitivity and dynamic range enables measurement of fluorescence excitation spectra at extremely low Chl concentrations; high temporal resolution; continuous monitoring of the light attenuation enables the use of an attenuation correction; storage of data in the submersible probe or on-line data transfer (via RS 485 intersection) to a personal computer (laptop PC) on board of, e.g., a research vessel; 'intelligent' data evaluation algorithm and user-friendly visualization software for personal computers. The submersible probe described above facilitates rapid depth-profiling of the phytoplankton population distribution; an example is shown in Fig. 4.

Fig. 3: Algal Chlorophyll-a is excited with light of five LEDs (emission wavelength 450 nm, 525 nm, 570 nm, 590 nm, 610 nm). The LEDs are switched alternately by a microcontroller. Chlorophyll-a fluorescence with wavelengths between 690 nm and 710 nm is detected by a photomultiplier-tube and the data is sent to the microcontroller. Data might be stored in the probe or transferred via RS 485 to a PC. A covering prevents the incidence of direct sunlight which might cause a perturbation of the measurement.
Measurement of the pressure enables the calculation of water-depths. An iterative gaussian fit weighted with the standard deviations of the norm spectra facilitates the estimation of the distribution of the spectral groups. The result is given in µg Chl a per L of the water sample



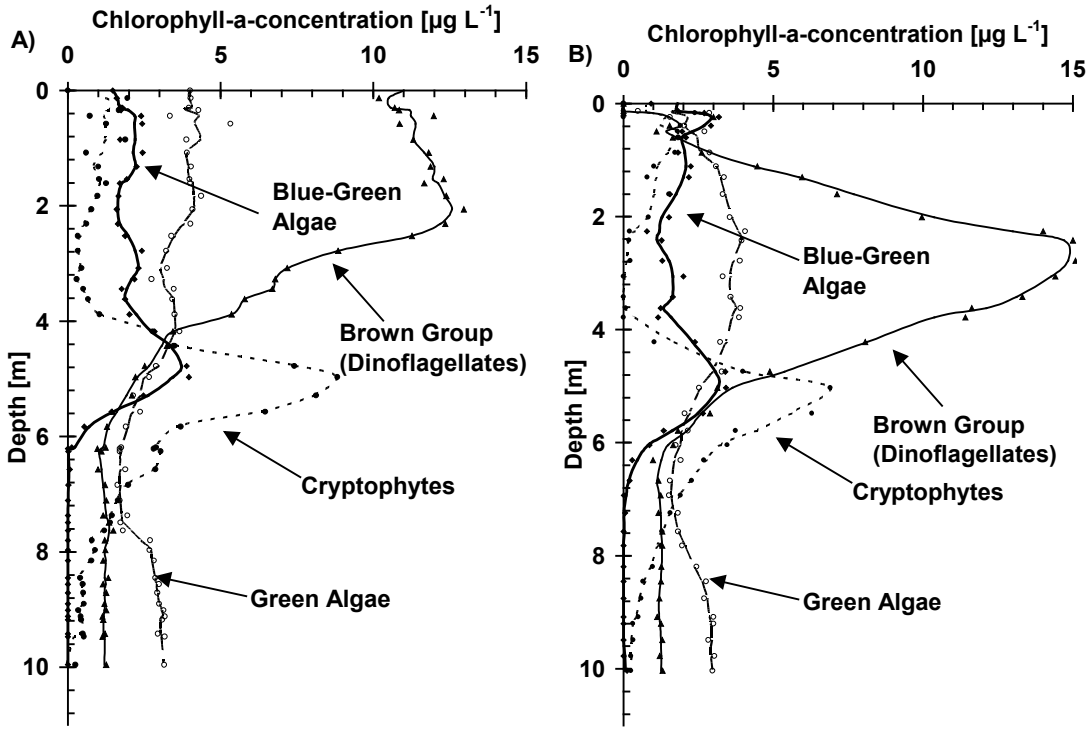


Fig. 4: Example of distribution profiles recorded with the submersible probe in Lake Plußsee (Northern Germany) at 04.08.98 showing vertical migration of dinoflagellates. Both measurements were made at the same location at 9:30 *am* and 2:00 *p.*m. The phytoplankton consisted of dinoflagellates (*Ceratium* spp.), chlorophyta (*Phacotus* sp.), blue-green algae/cyanobacteria (*Microcystis* spp., *Anabaena* spp.) and cryptophyta (*Cryptomonas* spp.). Dinoflagellates were dominating. At 9:30 *am* most of the dinoflagellates are situated at the surface (0 m - 2 m) of the lake. A maximum for cryptophyta was found at circa 5 metres.
At 2:00 *p.m.* the dinoflagellates were moving downwards in water layers with higher nutrient concentrations. Their maximum concentration could be found at 3 m depth while the other algal groups did not migrate.

In conclusion**,** the newly-developed bbe FluoroProbe facilitates assessment of algal population distribution with presently unsurpassed spatial and temporal resolution. For many investigations, the resolution of 3 or 4 algal groups should be sufficient. The accuracy of the algal-group differentiation is limited by the species-dependent variability within an individual algal group (see Fig. 2) and by the influence of environmental factors on the fluorescence yield (e.g., an inaccuracy of about 10% due to photoinhibitory fluorescence quenching is possible, data not shown). The bbe FluoroProbe will become an important new tool in aquatic ecology and for supervision of aquatic resources. With other methods not feasible investigations (or supervision tasks) may become manageable.