Remote Measurements of Chlorophyll a and Gelbstoff for Classifying Tidal Flats by Means of Laser Fluorosensors

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ABSTRACTS

With respect to the ecological importance and necessity of setting up a monitoring programme of the Wadden Sea, in vitro and in vivo fluorescence spectra of water and sediments in the Wadden Sea have been analyzed with the emphasis on chlorophyll a and gelbstoff fluorescence in Wadden Sea sediments. An algorithm is proposed that takes into account the self-absorption and the upward movement of benthic diatoms. This algorithm is applied in a quantitative determination of chlorophyll a using a laser fluorosensor, including the establishment of a scheme for classifying tidal flats by combining chlorophyll a and gelbstoff fluorescence. Results of in vivo fluorescence measurements acquired with a mini - laser fluorosensor in the Wadden Sea model are described. The in vivo fluorescence spectra and ground truth data are reported which were obtained by means of a shipboard and an airborne laser fluorosensor as well as ground sampling during two campaigns on the research ship TERRAMARE I to Crildumersiel.

1. INTRODUCTION

What is the current status of the Wadden Sea? Has it changed as a result of anthropogenic activities? Is the quality of the Wadden Sea getting better or worse? In order to partially answer these questions, a knowledge of the biomass and nutrient supplies, and consequently, a related long-term, large-area programme of monitoring the ecosystem Wadden Sea are necessary. With respect to the size and variability of the Wadden Sea in space and time, however, monitoring requires a practical approach other than sampling on foot or by ship (Doerffer et al., 1989; Reise, 1989).

Satellite imagery and aerial photography have been used by several authors with some success in supporting programmes of mapping the Wadden Sea (Doerffer et al., 1988; Fischer et al., 1993 & 1988). The micro-vegetation was mapped using aerial photography by Michaelis without positive results (Michaelis et al., 1982). A recent investigation of the macrophytobenthos in the Wadden Sea was mentioned by Doerffer and Murphy (Doerffer et al., 1989). The authors used the aerial data for visual classifications of macrophytobenthos in selected tidal areas. They are continuing this work and are trying to use their classification methods to study the microphytobenthos on the Wadden Sea surface (Murphy, 1991). The difficulty in investigating aerial photographs is that the colour of the Wadden Sea surface does not always correlate with the quantity of phytobenthic biomass, which results in difficulties in classifying tidal flats and estimating phytobenthic biomass by means of photogrammetry.

Since 1986 two Dornier DO 28 D2 surveillance aircraft have been flown in the German territorial areas of the North Sea and the Baltic Sea. In 1991 a new DO 228-212 aircraft with improved performance was put into operation (Grüner et al., 1991). The installation of an operational laser fluorosensor in the aircraft, which will qualify for continuous operation in maritime surveillance, is anticipated (Hengstermann et al., 1992b, Reuter et al., 1993). This laser fluorosensor is considered to be also capable of monitoring biological parameters and processes in the Wadden Sea over several days, seasons or years. It is thus expected that this sensor package can also be applied in remote fluorosensing of benthic

diatoms in Wadden Sea sediments as one of many continuous operations and as a long-term, large-area programme of monitoring the Wadden Sea ecosystem.

In order to realize such a programme, we think that: 1) Systematic spectral measurements and analysis of substances specifically present in the Wadden Sea are necessary if a laser fluorosensor or an imaging spectrometer is applied to investigate this coastal area; 2) An algorithm is required to estimate the phytobenthic biomass and vertical nutrient efflux in sediments on the basis of chlorophyll a and gelbstoff measurements; 3) A method has to be evaluated for calibrating the laser fluorosensor using ground truth measurements, including investigation on the influence of the fluorescence self-quenching effect and the upward movement of benthic diatoms on the phytobenthic biomass determination; 4) Experiments should be carried out to check whether it is possible to detect benthic diatom assemblages and indicate the nutrient efflux in Wadden Sea sediments by measuring in vivo chlorophyll a and gelbstoff fluorescence with a laser fluorosensor; 5) A programme has to be defined and established in an attempt to monitor the Wadden Sea by means of an airborne laser fluorosensor.

Some of these objectives are treated by Hengstermann et al. (1992a) and Wang (1992). This paper summarizes the experimental results of the feasibility studies on establishing a long-term, large-area programme for monitoring the Wadden Sea ecosystem by means of a laser fluorosensor.

2. SPECTROSCOPIC CHARACTERISTICS OF SUBSTANCES IN THE WADDEN SEA

Typical fluorescence spectra of substances in the Wadden Sea are summarized in Fig. 1 (Wang, 1992). The most obvious difference between the fluorescence spectra of the Wadden Sea and the open seawater is the relative change in the water Raman scattering at 381 nm when an excitation of 337 nm is used. The Raman intensity decreases and even disappears if turbid coastal water is considered. Compared with the fluorescence of open sea water, the gelbstoff fluorescence of the Wadden Sea increases at about 430 nm. This can be attributed to the high gelbstoff content in this area. There is also chlorophyll a fluorescence when algae are present in the sea water. Chlorophyll a fluorescence becomes more obvious in the fluorescence spectra of Wadden Sea sediments, in particular the peak at 735 nm, which is dominant when benthic diatom assemblages accumulate on the surface of Wadden Sea sediments. Such an obvious difference between the fluorescence spectra of Wadden Sea sediments with benthic diatom assemblages and those without them provides an opportunity to classify the Wadden Sea surfaces by means of the fluorescence technique.

2.1 Self-Absorption of Chlorophyll a

With respect to Fig. 1, there is usually a chlorophyll a fluorescence peak at a wavelength of 685 nm (abbreviated to F_{685}) in fluorescence spectra of open sea water with natural populations of algae. Another chlorophyll a fluorescence peak at about 735 nm (noted as F_{735}) is observed in the fluorescence spectra of Wadden Sea sediments. The second peak rises if the chlorophyll a concentration in sediments increases, for example in the case of benthic diatom assemblages growing on sediments. This second peak is comparable with the first one when optically thick diatom suspensions or high chlorophyll a concentrations are concerned (Wang, 1992). These two peaks, which may be attributed to the photosystem I and II, respectively (Murata et al, 1986), increase as the chlorophyll a content increases.

A fluorescence study of higher plants shows that chlorophyll a reabsorbs its fluorescence at about 685 nm and shows a strong fluorescence intensity at about 735 nm at high concentrations (Belanger et al, 1988, Lichtenthaler, 1986). A very thick film of benthic diatom assemblages on the Wadden Sea surfaces is one example of such a condition (Wang, 1992). This means that F_{735} increases when the high chlorophyll a concentration in benthic diatom assemblages is concerned. Compared with F_{735}, F_{685} increases relatively slowly if the high chlorophyll a concentration increases. This indicates that it is possible to use the F_{735}/F_{685} ratio to estimate the chlorophyll a content of thick benthic diatom assemblages in Wadden Sea sediments. This is usually done when a green plant stress is monitored (Lichtenthaler, 1988).

The fluorescence self-quenching effect of the chlorophyll a reabsorption can be explained more clearly by analyzing the two groups of the fluorescence spectra obtained using

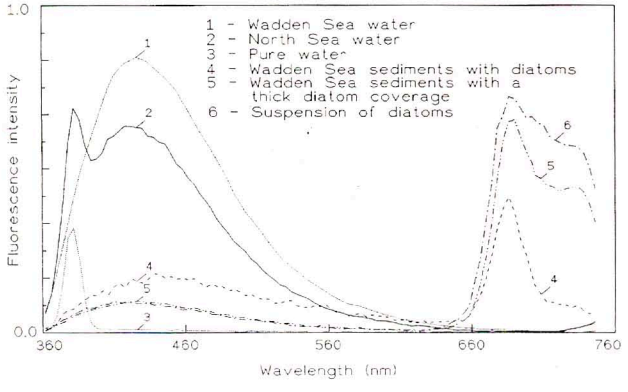

Fig. 1 - Typical fluorescence spectra of Wadden Sea water, North Sea water, purified water (produced by a SeraDest SP100 water purification system), Wadden Sea sediments, a diatom suspension and a film of benthic diatom assemblages on the surface of Wadden Sea sediments. An excitation of 337 nm was used.

the apparatus in Fig. 2a (see also table 1). In order to measure the reabsorption effect of samples in the first group with sample numbers 1 to 9 in the second cuvette, the first cuvette was filled with the chlorophyll a solution at a concentration of 2.7 mg/l which produces a constant fluorescence passing through the second cuvette. For the samples in the second group with sample numbers 10 to 19, the chlorophyll a solution at a concentration of 7.0 mg/l was filled into the first cuvette. It can be deduced from table 1 that the chlorophyll a reabsorption at about 685 nm results in a fluorescence decrease at about 685 nm and a wavelength shift corresponding to the fluorescence maximum, and a slight increase of the 735 nm fluorescence which is probably due to the second fluorescence caused by the chlorophyll a self-absorption of its fluorescence at about 685 nm.

Table 1 also shows that high chlorophyll a concentrations result in a fluorescence maximum at around 690 nm, while samples with low chlorophyll a concentrations have their maxima at about 680 nm. This is the reason why botanists always use the fluorescence at 690 nm, while oceanographers deal with the fluorescence at 685 nm or even 680 nm.

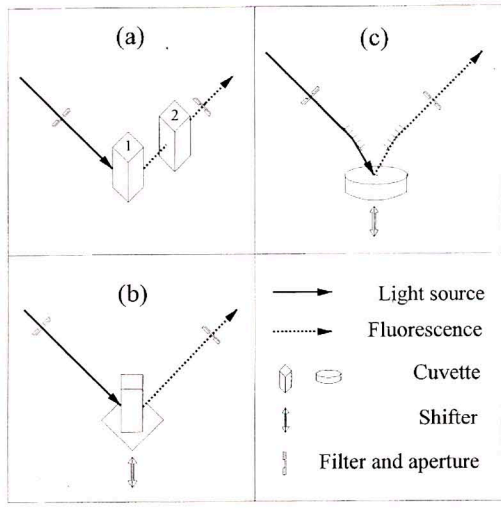

Fig. 2 - Modifications of the sample compartment of the Perkin Elmer fluorometer for measuring attenuation and reabsorption of the diatom suspension and chlorophyll a solutions (a), for measuring the upward and downward migration of benthic diatoms (b), and for measuring the fluorescence of sample surfaces (c). The arrangement (a) was used to demonstrate the self-absorption effect, while the arrangement (c) describes the configuration of laser fluorosensors applied in measuring fluorescence of the Wadden Sea surface.

Table 1: Spectroscopic characteristics of chlorophyll a solutions (in methanol). For comparison, a total of 19 samples are divided into two groups with sample numbers 1 to 9 and 10 to 19. Average value of the spectral parameters within a band width of 10 to 20 nm was taken to ensure the robustness of data and in consideration of the filters used in laser fluorosensors.

Sample	Concentration (g/l)	E_{685} (1/cm)	F_{685} (F'_{685}) (%)	$F_{735\ max}$ (%)	λ_{max} (nm)
1	0.60×10^{-7}		6.92	1.37	676.3
2	0.36×10^{-6}		6.73	1.34	676.5
3	0.21×10^{-5}		6.91	1.37	675.0
4	0.12×10^{-4}		6.69	1.31	675.0
5	0.78×10^{-4}	0.02	6.87 (6.90)	1.37	675.3
6	0.46×10^{-3}	0.04	6.51 (6.81)	1.33	678.0
7*	0.27×10^{-2}	0.15	5.82 (6.75)	1.38	679.0
8	0.17×10^{-1}	0.56	2.82 (6.80)	1.40	686.2
9	0.10	1.86	2.57 (6.78)	1.38	697.8
10	0.15×10^{-4}		9.56	2.26	679.5
11	0.39×10^{-4}		9.71	2.31	678.5
12	0.10×10^{-3}		9.54	2.31	679.5
13	0.28×10^{-3}	0.01	9.30	2.29	678.5
14	0.57×10^{-3}	0.05	9.14 (9.40)	2.31	679.3
15	0.13×10^{-2}	0.08	8.62 (9.61)	2.31	679.5
16	0.36×10^{-2}	0.15	7.67 (9.72)	2.28	680.8
17+	0.70×10^{-2}	0.27	6.56 (9.73)	2.32	683.3
18	0.19×10^{-1}	0.56	4.21 (9.66)	2.35	687.2
19	0.05	1.15	2.22 (9.73)	2.37	689.7

* In order to investigate the fluorescence self-absorption of chlorophyll a in the first sample group, this sample was used as a fluorescence sample which was put in the position of the scattering plate in Fig. 2a.

\+ This sample was used as a fluorescence sample in the second sample group.

The relative error becomes noticeable with high chlorophyll a concentrations (Fig. 3), indicating the necessity of correcting the chlorophyll a self-absorption at high concentrations.

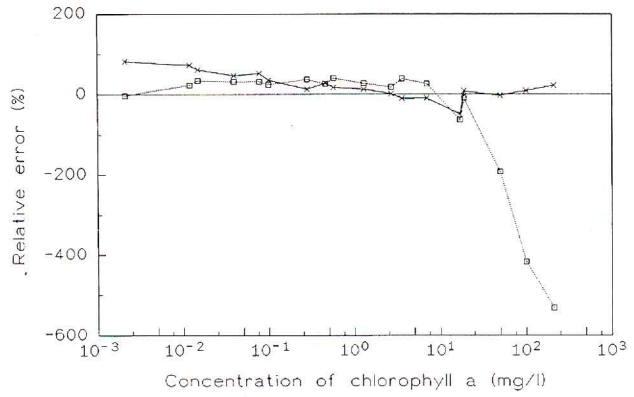

Fig. 3 - Relative error analysis of samples in table 1 for estimating chlorophyll a concentrations using fluorescence at 685 nm. Because of an increasing self-quenching effect of chlorophyll a fluorescence, the relative error increases with the chlorophyll a concentration if a linear model is used (rectangles). Much improvement can be achieved by correcting the reabsorption using a nonlinear model in Eq. (6) (crosses, see Fig. 7)). Further explanations are given in the text.

2.2 Influence of water coverage on the sediment fluorescence

Water coverage on sediment surfaces essentially changesthe fluorescence signatures of sediments (Hengstermann et al, 1992a; Wang, 1992). There is a tendency of increasing water coverage to increase gelbstoff fluorescence, as chlorophyll a fluorescence decreases. In an extreme case the gelbstoff fluorescence can be so dominant that chlorophyll a fluorescence virtually disappears. This suggests a way in which the fluorescence relationship between gelbstoff and chlorophyll a fluorescence can be estimated to classify the Wadden Sea according to the depth of a layer of water on sediments.

2.3 Fluorescence change due to the upward movement of benthic diatoms

In tidal areas, benthic diatoms migrate very quickly from deeper sediment layers to the surface under favourable conditions of light, temperature, and moisture content (G. Cadee, 1984). Stratingh et al. (1855) were probably the first to observe that the mudflats during low tide "bloomed" in spring with the formation of a brown surface film which could be peeled off by the incoming tide. They were, however, not aware of the fact that benthic diatom assemblages caused this bloom, although they mentioned eight species of benthic diatoms found in the Dollard.

In proof of the movement of benthic diatoms, Fig. 4 shows that the originally white filters (lens tissue, Wang, 1992) become brown after two hours of exposure on the sediment surface and that the films of benthic diatom assemblages are peeled off by the incoming tide.

Laboratory studies of the upward movement of benthic diatoms were made by Wang (1992) using the apparatus in Fig. 2b. The cuvette was put under a vertically illuminating light field at room temperature and laterally excited with an excitation light beam of about 0.2 mm in height. In this way, the fluorescence emitted from different sediment layers was measured by adjusting the cuvette upwards and downwards. It was found that the chlorophyll a fluorescence of sediment surfaces increases as the sample is continuously illuminated, indicating the upward movement of benthic diatoms. The natural downward migration of benthic diatoms was not observed during the experiments in the laboratory, even though diurnal measurements were done. It is suspected that the downward movement of benthic diatoms may occur when sediment surfaces are too dry and in the case of nutrient deficiency. Such is usually the case in the Wadden Sea sediments, because the sediments become dry and harder following low water. This results in pieces of thin films of benthic diatom assemblages being peeled off by the incoming tide and floating on the water surface (Fig. 4).

3. REMOTE SENSING OF SUBSTANCES IN THE WADDEN SEA USING A LASER FLUOROSENSOR

3.1 A model of three fluorescence parameters

Chlorophyll a at a concentration of up to 20 $\mu g/cm^2$ in Wadden Sea sediments is nearly of the same order as that found in the leaves of green plants. Such high chlorophyll a concentrations produce strong fluorescence at both 685 and 735 nm and make the fluorescence self-quenching noticeable (Lichtenthaler, 1988), resulting in a large relative error for an estimate of chlorophyll a concentrations using the fluorescence at 685 nm (Fig. 2). This means that the fluorescence intensity at about 685 nm is not proportional to the chlorophyll a concentration and the chlorophyll a fluorescence at about 735 nm may be used as an additional parameter for determining chlorophyll a concentrations and for assessing biomass on Wadden Sea sediments. Apart from this, Wadden Sea sediments contain not only chlorophyll a but also gelbstoff and particles. This results in both the absorption and fluorescence of one component being influenced by the other two components. Another factor that should be taken into account is the upward movement of

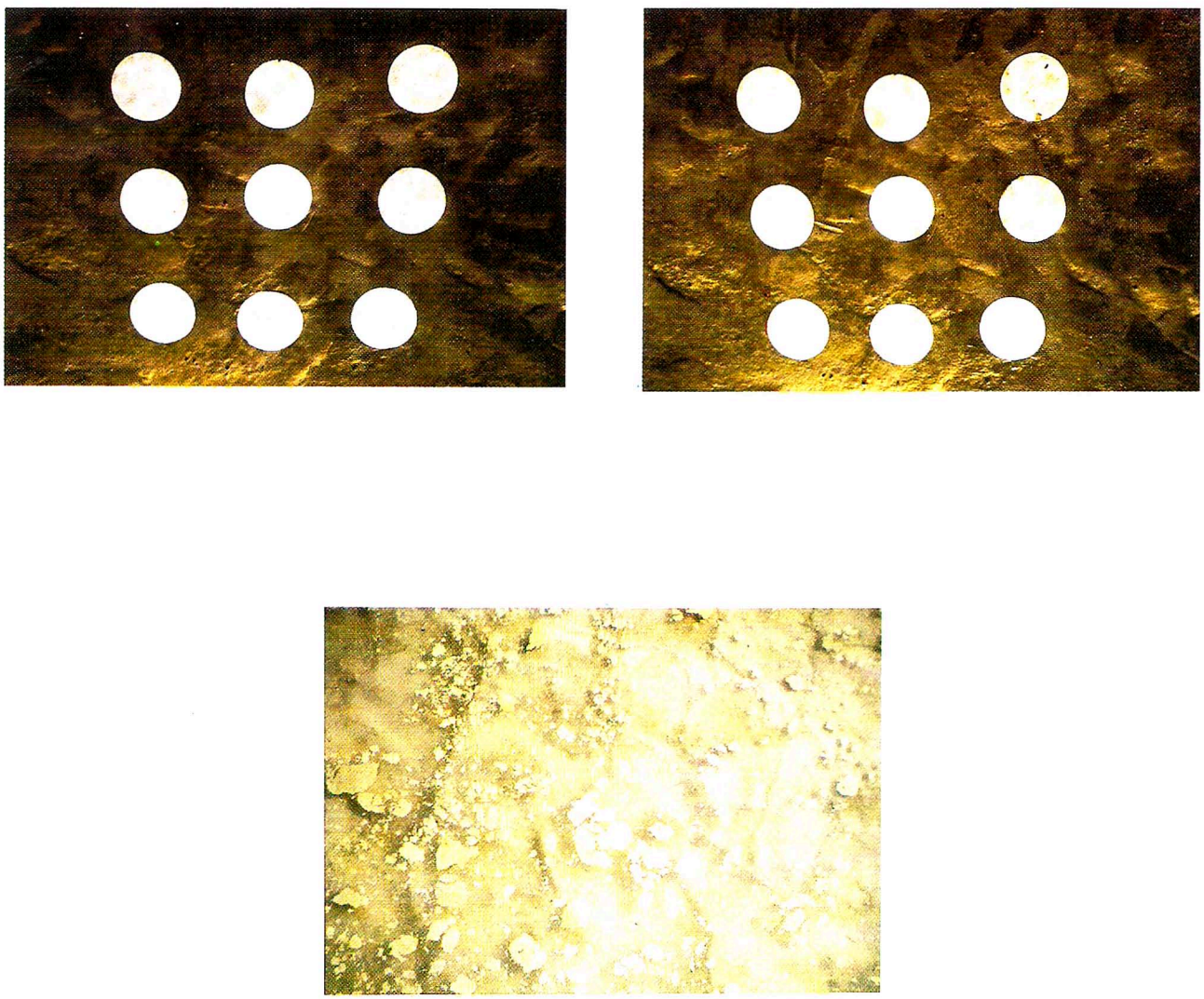

Fig. 4 - Photographs of benthic diatom assemblages on surfaces of the Wadden Sea sediment and incoming tidal water, taken just at the beginning of the ebb (**upper left**), *two hours later* (**upper right**) *and during flood* (**lower part**). *Filters were used as lens tissues to collect diatoms. (Taken during the trip on 15th of October 1991, at Crildumersiel, 53° 40.13'N, 08° 03.71'E). 9 filters were used for one sample to avoid inhomogeneity of the benthic diatom assemblages collected.*

benthic diatoms, which causes an increase of chlorophyll a fluorescence as these diatoms accumulate on the sediment surface. To quantitatively determine the chlorophyll a concentration in benthic diatoms by means of laser-induced fluorescence, an algorithm is therefore needed that takes into account corrections of the fluorescence self-quenching effect and the diatoms' upward movement, together with interactions between the two photosystems of chlorophyll a and interrelated influences among other components in Wadden Sea sediments.

In our case, the fluorescence of the Wadden Sea sediments can be generalized and symbolically expressed as follows if the fluorescence spectra of a mixture of chlorophyll a with gelbstoff are taken into account (Wang, 1992):

$$F_\lambda = A \bullet B_{\lambda e}\,(\text{chlorophyll a}, \text{gelbstoff}) \otimes (B'_\lambda \otimes F_\lambda) \quad (1)$$

$\otimes$ represents a mathematical and symbolical interaction operator;

$B_{\lambda e}$ is the competing interaction between absorptions of chlorophyll a and gelbstoff, meaning for example, that the measured fluorescence F_λ of chlorophyll a in a mixture is equal to the fluorescence F'_λ of pure chlorophyll a operated ($\otimes$) by a factor which impairs chlorophyll a absorption due to the coexistence of gelbstoff;

B'_λ is the influence of a factor such as the self-quenching effect on the measured fluorescence F_λ;

A is the constant relevant to instrument parameters, etc.

Eq. (1) includes the fluorescence self-quenching effect and specifically for the case of Wadden Sea sediments, where only gelbstoff and chlorophyll a are mixed, as revealed by in vitro fluorescence measured in the laboratories (Hengstermann et al., 1992a). With respect to the different absorption coefficients of gelbstoff and chlorophyll a at 337 nm, chlorophyll a self-absorption at 685 nm, and the contribution of the PS II fluorescence to the PS I fluorescence at 735 nm, Eq. (1) can be corrected as follows if a fluorescence spectrum at three wavelengths is considered:

$$F_{450} = A \bullet B_{337}\,(\text{chlorophyll a}) \otimes (1 - E_{450}) \otimes F'_{450} \quad (2)$$

$$F_{685} = A \bullet B_{337}\,(\text{gelbstoff}) \otimes (1 - E_{685}) \otimes F'_{685} \quad (3)$$

$$F_{735} = A \bullet B_{337}\,(\text{gelbstoff}) \otimes (1 - E_{735}) \otimes (F'_{735} + D \bullet F_{685}) \quad (4)$$

Here:

F'_{450} and F_{450} are the true and the measured gelbstoff fluorescence intensities at 450 nm;

B_{337} (chlorophyll a) is the correction factor for the light intensity absorbed by gelbstoff due to the competing chlorophyll a absorption;

E_{450} is the absorption coefficients of chlorophyll a and gelbstoff at 450 nm;

F'_{685} and F_{685} are the true and the measured chlorophyll a fluorescence intensities at 685 nm;

B_{337} (gelbstoff) is the correction factor for the light intensity absorbed by chlorophyll a due to the competing gelbstoff absorption.

E_{685} is the absorption coefficient of chlorophyll a at 685 nm; the gelbstoff absorption at this wavelength is negligible;

E_{735} are the absorption coefficients of chlorophyll a and gelbstoff at 735 nm;

F'_{735} and F_{735} are the true and the measured chlorophyll a fluorescence intensities at 735 nm;

$D \bullet F''_{685}$ is the contribution of the PS II fluorescence to the PS I fluorescence at about 735 nm.

The fluorescence arising from internal interactions between photosystems, the fluorescence emission of PS I at about 685 nm and the second fluorescence at about 735 nm are not included here, because the PS I fluorescence at about 685 nm is negligible when compared with that of PS II. The internal interaction between photosystems may be spillover; for example, whose contribution to fluorescence emission is still not clear.

In practice, there are other components mixed with gelbstoff and chlorophyll a in Wadden Sea sediments which absorb laser light only and do not emit fluorescence. Sand, mud and non-chlorophyll a particles are such components. In addition to this, the fluorescence at 450 nm could be caused by gelbstoff both in sediments and in benthic diatoms. In such cases more correction factors should be included in Eqs. (2) to (4). A correction of the in vivo fluorescence should also be made if light and nutrient stresses exist in benthic diatoms (Kiefer et al., 1973). The contribution of pheophytin a fluorescence to the measured fluorescence should also be taken into account, because pheophytin a has nearly the same fluorescence spectrum as chlorophyll a (Wang et al., 1992b) and does not always correlate with chlorophyll a (Wolff, 1979).

Only the second fluorescence of PS I and the reabsorption of PS II at 658 nm are discussed in more detail in the

following section, because: 1). There are limits imposed by the experimental conditions; 2). These two effects make the chlorophyll a fluorescence at 735 nm comparable with that at 685 nm and both depend nonlinearly on the chlorophyll a concentration; 3). The other factors such as internal interaction between photosystems are still an open question and are being researched by physiological experts. It is also assumed that such internal interactions would not influence the chlorophyll a fluorescence very strongly; 4). Combined with the chlorophyll a fluorescence, the gelbstoff fluorescence at about 450 nm is used qualitatively in this thesis for classifying tidal flats because gelbstoff concentrations are not quantitatively determined by sampling; and 5). It will be shown later that the corrections for these two effects are good enough to determine the chlorophyll a concentration quantitatively, even though the concentration is very high.

All the above equations are applicable to dry-fallen Wadden Sea sediments. The influence of a water layer on the sediment fluorescence should be included when there is a thin water coverage. A water coverage attenuates not only the laser beam but also the sediment fluorescence. In addition to this, the upward movement of benthic diatoms increases the chlorophyll a fluorescence of sediments. This makes the correction factor $B_{\lambda e}$ in Eqs. (2) to (4) more complicated and is discussed by Wang (1992).

3.2 Scheme for classifying tidal flats

Equations (2) to (4) can be further combined according to the optical characteristics of the object detected and normalized as follows:

$$F_{450} + F_{685} + F_{735} = 1 \qquad (5)$$

The advantage of such a normalization in Eq. (5) is that fluorescence parameters reveal the change of spectral shapes and can be presented intuitively by a ternary diagram for a classification analysis (Aitchison, 1986). It is plausible that fluorescence spectra change their form if the depth of water coverages varies on sediment surfaces, and that fluorescence parameters are correlated with the chlorophyll a concentration.

Fig. 5 shows the fluorescence spectra of chlorophyll a at various concentrations in table 1 measured with the apparatus in Fig. 2c. The cuvette (5.0 cm in diameter and about 0.3 cm in depth) was filled with chlorophyll a solutions and simulated a film of benthic diatom assemblages in Wadden Sea sediments.

Fig. 6 combines the information indicated in Figs. 1 and 5 and in table 1, and takes into account the normalization in Eq. (5). Similar results have been obtained with Wadden Sea sediment samples using the apparatus in Fig. 2c. The sediment samples were supplied by biologists from the Department of Biology, University of Oldenburg, who took them from the tidal flats close to Wilhelmshaven.

Table 2 summarizes this information and presents a specific classification scheme for Wadden Sea water and sediments by means of laser fluorosensors.

I). Very strong gelbstoff fluorescence ($F_{450} \gg F_{685}$, $F_{735} \approx 0$).
An optically thick water column is detected, such as the open sea without algae, coastal water with a high gelbstoff concentration, etc.

II). Low chlorophyll a fluorescence and strong gelbstoff fluorescence ($F_{450} \geq F_{685}$, $F_{735} \approx 0$).
A thin water coverage on the sediments or a body of water with algae of natural populations are detected. The detected fluorescence is composed of organic matter and plankton as well as microphytobenthos.

III). Strong chlorophyll a fluorescence at about 685 nm and low gelbstoff fluorescence ($F_{450} < F_{685}$ and $(F_{735}/F_{685}) < 0.2$).
An alga bloom and Wadden Sea sediments with benthic diatoms in the open sea is detected.

Table 2: Scheme for classifying the Wadden Sea water and sediments by laser fluorosensors. Three fluorescence parameters are used and normalized as: $F_{450} + F_{685} + F_{735} = 1$.

Scheme	F_{450}	F_{685}	F_{735}	F_{685}/F_{450}	F_{735}/F_{685}
I	1	0	0		
II	0.5< & <1	<0.5	0	<1	
III	<1	<1	>0	>1*	<0.3
IV	<1	<1	<0.5+	>1*	<0.5
V	<1	<1	>0.5+	>1	>1
VI	<0.5	0.5< & <1	0.5< & <1	>1	>1

* It could be smaller than 1 if sediments with or without benthic diatom assemblages are covered by a thin water coverage.
+ A different boundary value may be required if different subjects are concerned (e.g. chlorophyll a solutions in table 1).

IV) Strong chlorophyll a fluorescence and low gelbstoff fluorescence ($F_{450}<F_{685}$ and $0<(F_{735}/F_{685})<0.5$).
Wadden Sea sediments with a visible film of benthic diatom assemblages are detected.
Schemes III and IV may also apply when Wadden Sea sediments with or without visible benthic diatom assemblages are covered by a thin layer of water. In these cases F_{450} may be larger than F_{685}.

V). Very strong chlorophyll a fluorescence and low gelbstoff fluorescence ($F_{450}<F_{685}$ and $0.5<(F_{735}/F_{685})$).
Wadden Sea sediments with a very thick film of benthic diatom assemblages are detected. This may occur at those places along the tidal inlets where sufficient moisture and nutrient effluxes in the sediments favour the growth of benthic diatoms, resulting in a dark brown sediment surface. This phenomena can usually also be observed in the fluorescence spectra of higher plants.

VI) Strong chlorophyll a fluorescence at about 735 nm and low gelbstoff fluorescence ($F_{450}<F_{685}$ and $1<(F_{735}/F_{685})$).
Chlorophyll a fluorescence at about 685 nm decreases as the chlorophyll a concentration increases. Such cases can be observed if very dense diatom suspensions or dark green leaves of higher plants are present.

3.3 Algorithm for measuring chlorophyll a concentration

Fig. 7 was obtained by measuring fluorescence spectra of all samples in table 1 using the apparatus shown in Fig. 2c. Due to the increasing self-quenching effect of chlorophyll a fluorescence at 658 nm, the relative error (rectangles in Fig. 3) increases with the chlorophyll a concentration if the linear dependence of the chlorophyll a concentration on the fluorescence at 685 nm (i.e. linear model) is taken into account.

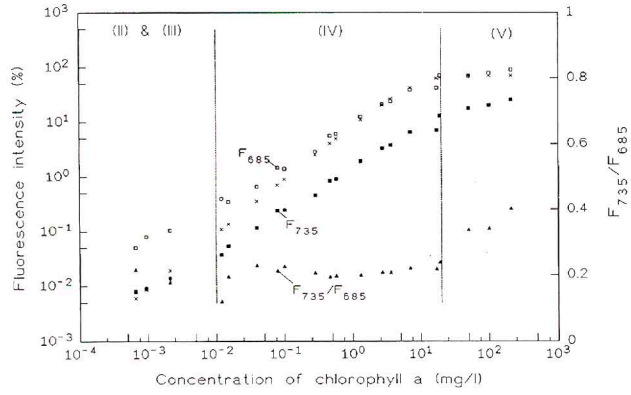

Fig. 7 - Change of the measured F_{685} (rectangles), F_{735} (solid rectangles) and their ratio (solid triangles) of chlorophyll a with the chlorophyll a concentration. This forms a basis for the classification of sediments. Crosses show the change of the F_{685} obtained according to Eq. (7), indicating a more precise determination of chlorophyll a concentrations (see Fig. 3). An excitation wavelength of 450 nm was used.

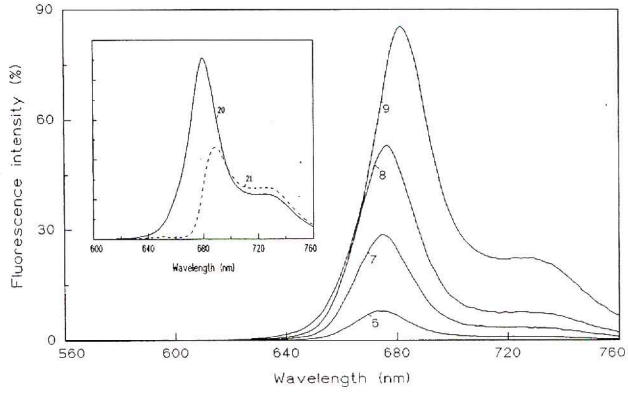

Fig. 5 - The measured chlorophyll a fluorescence spectra show the fluorescence-quenching effect due to high chlorophyll a concentrations. The line numbers are the sample numbers in table 1, with the exception of numbers 20 and 21 which are from two diatom suspension samples with chlorophyll a concentrations at about 10 mg/l and 1 g/l. For simplicity only the fluorescence spectra of samples in the first group in table 1 are shown. The spectra in the second group show the same effect as those in the first group. An excitation wavelength of 450 nm was used.

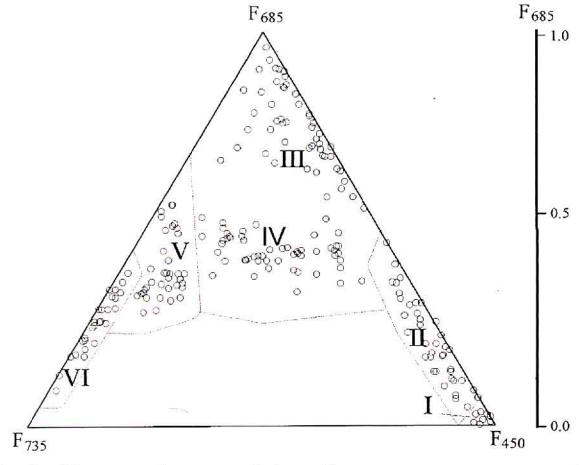

Fig. 6 - Ternary diagram of three fluorescence parameters for classifying tidal flats. A total of six classes are grouped. The circles show the data used in Figs. 1, 3 and 5. With the bisector used as an axis, each parameter in the figure has its maximum value 1 at the angle where the symbol of this parameter is written, and its minimum value 0.0 at the side opposite this angle (as indicated for example in the inset for F_{685} in the right-hand corner). For all parameters the normalization $F_{450} + F_{685} + F_{735} = 1$ is made.

Based on the transfer function of the laser fluorosensor and referring to Fig. 2c, the following equation can be derived to correct the self-absorption of chlorophyll a at 685 nm (nonlinear model, Wang, 1992).

$$F_{685} = A_0 \bullet \phi\ 337{,}685 \bullet \frac{1}{E_{337} + E_{685}} \bullet$$

$$\left[1 - \exp\left[-(E_{337} + E_{685}) \bullet d \right] \right] \qquad (6)$$

Laser excitation at 337 is used and $\phi_{337,685}$ represents the fluorescence conversion efficiency of chlorophyll a at 685 nm when excited at 337 nm. d in Eq. (6) is the thickness of the sample penetrated by the laser.

Eq. (6) can be simplified as follows by inserting the data in Fig. 7:

$$F_{685} = 70\ [1\text{-}\exp(-0.13\ C)] \qquad (7)$$

with C the chlorophyll a concentration (mg/l).

According to this equation chlorophyll a fluorescence can be computed taking into account the chlorophyll a self-absorption (shown in Fig. 7 by crosses). The relative errors relevant to this nonlinear model are shown in Fig. 3 by crosses. When comparing the small relative errors shown in crosses with those shown by rectangles, it is evident that many improvements can be achieved by correcting the chlorophyll a self-absorption using the nonlinear model in Eq. (7).

For simplicity the chlorophyll a solutions were used here to obtain Eq. (7). A similar behaviour is assumed to exist in living cells. Sediment samples were also measured and quantitatively discussed by Wang (1992).

4. FIELD EXPERIMENTS FOR FEASIBILITY STUDIES OF USING LASER FLUOROSENSORS FOR MONITORING THE WADDEN SEA

4.1 Laser fluorosensors

A mini-nitrogen laser-based fluorosensor (MiLF) has been developed to obtain field fluorescence spectra of Wadden Sea water and sediments. As shown in table 3, a monochromator was used as a spectroscope at the beginning of the experiments (Hengstermann et al., 1992a & b, Wang, 1992). Lateron the MiLF was modified and equipped with a spectroscope which consists of a group of dichromatic splitting mirrors and interference filters. The modified MiLF was equipped for simultaneously measuring fluorescence at the following wavelengths: 366, 450, 650, 685 and 735 nm (see table 3).
The MiLF was assembled in a large crate and was mobile when the experiments were done in the Wadden Sea model in a glasshouse. Lateron it was installed in a van during the outdoor campaigns in the tidal flats close to Crildumersiel.

Table 3 shows the LFS, which is an airborne laser fluorosensor and has been developed at the Department of Physics, University of Oldenburg (Hengstermann et al., 1992b). The LFS was installed in a DO 228 aircraft which was flown over the tidal flats near Crildumersiel during the low tide period of the second campaign on September 19, 1991.

4.2 Samples and parameters

Table 4.5 shows the samples taken and the parameters measured during the experiments done in the Wadden Sea model and on board the ship TERRAMARE I as well as on board the aircraft. Water, diatom suspensions, sediments

Table 3: Characteristics of the laser fluorosensors applied in the experiments done in the Wadden Sea model and during two campaigns later on (refer to Chapter 5).

laser fluorosensor	laser	spectrograph	sampling technique
MiLF	N_2 (337 nm)	scan in a range of 350 to nm	robust multi sample averaging[#]
Modified MiLF	N_2 (337 nm)	channels at 360 450, 600, 650, 685 and 735 nm	robust multi sample averaging[#]
LFS	Excimer[*] (308 nm)	channels at 332, 344, 366, 380, 410, 440, 470, 500, 550, 600, 685 and 735 mm	single sampling

[*] The use of a dye laser at 382 nm is possible in future (Reuter et al, 1993).
[#] Wang et al (1992).

Table 4: List of the samples taken and the parameters measured during the experiments done in the Wadden Sea model and on board the ship TERRAMARE I as well as on board the aircraft.

Samples	Method used	Parameters
Standard samples	Fluorometer	in vitro fluorescence and calibration
	Spectrophotometer	attenuation and pigment content
Sediments	Fluorometer	in vitro chlorophyll a fluorescence and determination of pigment contents
	Lens tissue technique	determination of pigment contents
	MiLF	in vivo chlorophyll a fluorescence and gelbstoff fluorescence
	Microscope*	cell observation and taxonomy
	LFS#	in vivo chlorophyll a fluorescence and gelbstoff fluorescence (wide-scale)
Water+		determination of nutrients

* It was done only during the "Crildumersiel I" campaign in August 13-16, 1991.
+ It was done only during the "Crildumersiel II" campaign on September 19, 1991.

Table 5: Correlation matrix between contents of chlorophyll a, pheophytin a and nutrients selected from the data in table 5.4 (n=6).

Sample	Chlorophyll a in		Pheophytin a in		Nutrients		
	LT#	SE*	LT#	SE*	NH_4^+	NO_2^-	NO_3^-
Chlorophyll a in							
LT#	1.00						
SE*	0.49	1.00					
Pheophytin a in							
LT#	0.97	0.29	1.00				
SE*	0.84	0.42	0.88	1.00			
Nutrients							
NH_4^+	0.81	0.00	0.82	0.46	1.00		
NO_2^-	-0.58	0.15	-0.75	-0.83	-0.44	1.00	
NO_3^-	-0.88	-0.74	-0.83	-0.92	-0.44	0.55	1.00

short form of lens tissue (Lorenzen, 1966; Wonnenberger et al, 1984; Stal et al, 1984).
* Short form for sediment layer of top 0.5 cm.

and chlorophylls, which were prepared according to Wang (1992), are standard samples.

4.3 Laser fluorosensing measurements in the Wadden Sea model

Fluorescence spectra were measured using the MiLF in both basin 1 and basin 2 of the Wadden Sea model in a glasshouse laboratory (Wang, 1992), since there was a visible film of benthic diatom assemblages on the sediment surface found in basin 1, and there were no benthic diatom assemblages observed in basin 2. This means that basin 1 is productive and basin 2 simulates a tidal flat without benthic diatoms.

Results almost the same as those mentioned in Chapter 2 were obtained when the MiLF was used in the Wadden Sea model. With the MiLF it was possible to measure the fluorescence of the Wadden Sea without disturbing it. This is certainly very important, because any touching of the sediments may result in a change of distribution of benthic diatom assemblages.

Fig. 8 shows the fluorescence spectra which were obtained during a tidal cycle. This ternary diagram is produced by means of the normalization in Eq. (5) (Aitchison, 1986). The circles show the fluorescence spectra which were obtained during flood in basin 2, while the triangles show the spectra for basin 1. The water level increases with the number of spectra. It can be deduced that an increase of gelbstoff fluorescence results in a decrease of chlorophyll a fluorescence, accompanied by an increase of the water level on the sediment surface. In an extreme case the gelbstoff fluorescence can be so dominant that chlorophyll a fluorescence virtually disappears, because the 337 nm laser light is attenuated to zero at the sediment surface by the water coverage (e.g. spectral numbers 4 and 5 in Fig. 8).

This corresponds to scheme II in table 2 and suggests a way in which a relationship between gelbstoff and chlorophyll a fluorescence can be revealed. This relation can be used to classify the Wadden Sea surface. Referring to Fig. 8, basins 1 and 2 can be distinguished even if there is a thin layer of water covering the sediment surface. As described at the beginning of this section, there was a visible film of benthic diatom assemblages on the sediment surface of basin 1 and no benthic diatom assemblages were observed in basin 2. It was found that the chlorophyll a concentration was 0.85 $\mu g/cm^2$ in basin 1 and 0.12 $\mu g/cm^2$ in basin 2. The absorption coefficient of water at a wavelength of 337 nm in both basins was about 2 cm^{-1}. It is therefore to be expected that there is a strong chlorophyll a fluorescence in basin 1 and that a coverage of a 1 cm water layer attenuates the laser light to zero, leading to there being no light available to excite the chlorophyll a in the sediments (covered by a 1 cm water layer) to produce fluorescence.

Due to high gelbstoff concentrations in the Wadden Sea water, no straightforward classification of the body of water and sediments with water coverage in different basins is possible. However, it is possible to classify the sediments with very thin water coverages by using multivariate statistics (Hengstermann et al.,1992a).

4.4 Campaign "Crildumersiel I"

Description of the campaign

On August 13, 1991 the research ship TERRAMARE I anchored at a position of 53° 40.13'N, 08° 03.71'E. As shown in Fig. 9, the area consists of two tidal flats: One sandflat is bounded by the main tidal inlet on the west side and the deep water line on the east side (Reichardt, 1980); the other flat on the east side of the main tidal inlet consists of sand and mud on which inhomogeneous benthic diatom assemblages were observed. This area was selected, because it has been used for experiments by biologists for a long time (Langner van Voorst, 1991). For comparison, sample positions 1 and 3 were selected individually on the sandflats and sand-mudflats, and sample position 2 was in between these two flats.

During the trip the in vivo fluorescence spectra of benthic diatoms in Wadden Sea sediments were measured using a

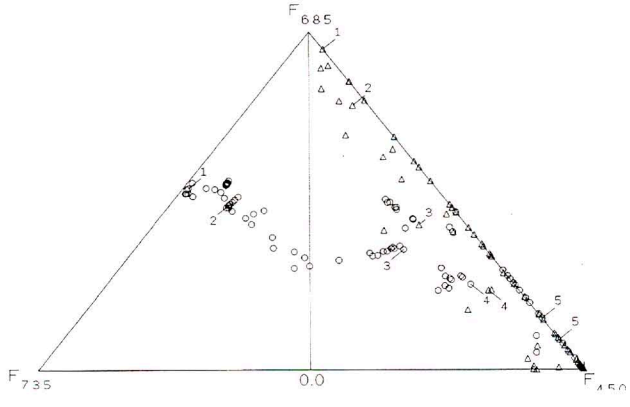

Fig. 8 - Ternary diagram for fluorescence parameters of sediments of the Wadden Sea model. The spectra were measured during flood (ebb - flood) in basin 1 (circles) and basin 2 (triangles), respectively. The number of data points increases along the line connecting F_{685} to F_{450}. This tendency is correlated with an increasing water level above the sediment surface. See the explanation of Fig. 6 for the axis of each parameter.

laser fluorosensor (MiLF). As described above, the MiLF was installed in a van which was fixed on board the ship. The laser was directed onto the sediment surface by a large mirror which also reflected the fluorescence from sediments onto the telescope. An estimation of the pigment in the top sediment layer was performed by taking sediment samples and using the lens tissue technique (see table 4).

Pigment distributions

Concentrations of chlorophyll a and pheophytin a at various sample positions were determined both by using the lens tissue technique and taking sediment samples (Höpner et al., 1983; Stal et al., 1984; Wang, 1992). The concentrations of bacteriochlorophyll a and bacteriopheophytin a were also determined and found to be so low that they are negligible. The samples taken at position 1 contain relatively lower chlorophyll a and pheophytin a concentrations than those taken at position 3, which indicates lower quantities of benthic diatom assemblages in the sandflats than in the sand-mudflats (see Fig. 9). It was observed during the experiments that the surfaces of sand-mudflats turned a darker brown than those of sandflats.

The correlation analysis shows that chlorophyll a and pheophytin a have a positive correlation in the lens tissue, but a negative correlation in the sediment. This even holds true when the data are divided into three groups according to the date of measurements. The reason is assumed to be the high pheophytin a concentration below the upper layer of sediments, which was found when sediments were taken, but which was not found when the lens tissue technique was used.

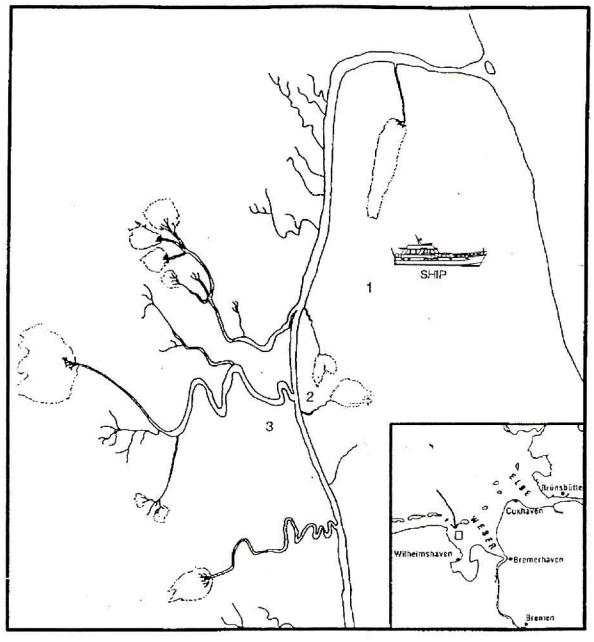

Fig. 9 - Location of the experimental area and distribution of sample positions 1, 2 and 3. The ships position was 53° 40.13'N, 08° 03.71'E. The main tidal inlet here corresponds to the tidal inlet II in Fig. 12. This map was made in 1990 by van Voorst with the help of aerial photographs.

Analysis of in vivo fluorescence

Fig. 10 shows the typical fluorescence spectra of Wadden Sea sediments obtained using the MiLF. From time to time benthic diatoms creep up from the deeper layer to the surface of the sediments. This results in an increase of density of benthic diatom assemblages on the sediment surface and thus in an increase of fluorescence (Fig. 11). Some time later the chlorophyll a fluorescence of Wadden Sea sediments decreases. This may be due to the downward migration, the death of benthic diatoms, and probably the appearance of nutrients and water as well as light stress (photoinhibition). It has been reported that the in vivo fluorescence of diatoms declines when continuously exposed to intense light and that diatoms leave behind them invisible traces when they move (Kiefer, 1973, Hopkins, 1967). Downward movements of benthic diatoms leave organic matter on the surface of Wadden sea sediments. It was observed that the sediment surface becomes dark brown and dry, and shows an increase of gelbstoff fluorescence when strongly illuminated.
Fig. 11 also shows the importance of correcting the self-quenching effect of chlorophyll a fluorescence, because a better correlation between F_{735} and F_{685} is found after the correction of the chlorophyll a reabsorption at 685 nm if the measured data (circles) are compared with the computed data according to the nonlinear model (crosses). The corrections were made for the data points where F_{735}/F_{685} is larger than 0.5.

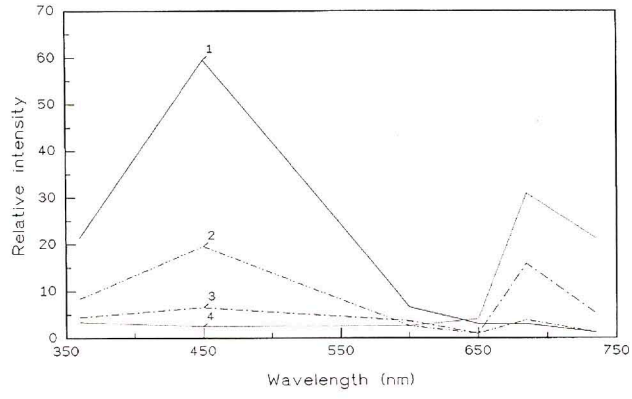

Fig. 10 - Typical fluorescence spectra measured with the MiLF (λ_{ex} = 337 nm): Sediments with optically thick water coverages (1), sediments with thin water coverages (2), sediments (3) and sediments with a visible film of benthic diatom assemblages (4).

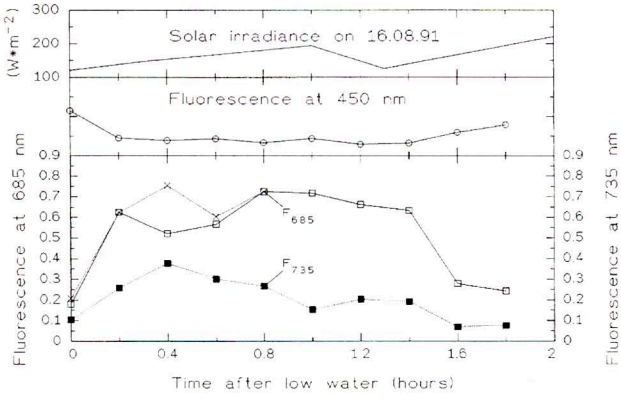

*Fig. 11 - Change of the Wadden Sea sediment fluorescence at various times (***lower part***). The sediment surface was covered by different amounts of benthic diatom assemblages as revealed by the change of chlorophyll a fluorescence intensities. The crosses show the fluorescence intensities of chlorophyll a after the correction for the chlorophyll a self-absorption at about 658 nm. The* **middle section** *of this figure shows the change of gelbstoff fluorescence and the* **upper section** *shows the change of the solar irradiance during the experiments.*

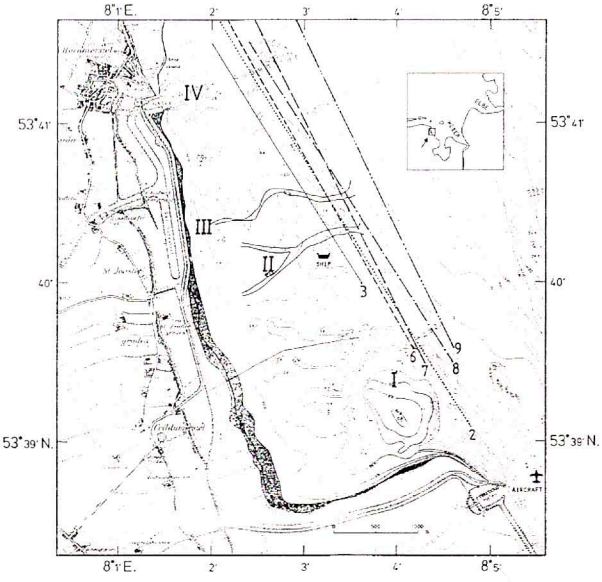

Fig. 12 - Location of the experimental area and distribution of samples. The lines show the flight tracks during the experiments (except flight tracks 2 and 9 which were individually cut off at the beginning of flight 2 and at the end of flight 9, see table 5.3 for the positions of flight tracks). The map was made by Kuratorium für Forschung im Küsteningenieurwesen (Kiel, Germany) in 1976 and is the latest available. Tidal inlets II and III were drawn on the basis of observation and photographs. It was observed that tidal inlets I, II, III and IV were about 70, 8, 5 and 70 m wide, respectively. All tidal inlets became narrow when the latitude decreased.

Correlation analysis shows that the in vivo fluorescence at 685 nm correlates better and more stably with the chlorophyll a content in the top 1 cm layer of sediments than that in lens tissues. This is due to the fact that only motile diatoms were collected in the lens tissues, while in vivo fluorescence measured using laser fluorosensors arises from all benthic diatoms in sediments. It is therefore recommended that the chlorophyll a content in sediments is used to calibrate laser fluorosensors.

4.5 "Cridumersiel II" Campaign

Description of the campaign

As shown in Fig. 12, on September 19, 1991 the research ship TERRAMARE I again anchored at the position described in Fig. 9. The experiments were performed at ebb from about 15:00 to 17:30. A film of benthic diatom assemblages was observed which was less brown than that found during the first trip. This was due to bad weather conditions (cloudy, raining) during the second trip.

During the trip the MiLF was operated as described above. The LFS was installed in a DO 228 aircraft which flew over the experiment area during low tide. A total of six flights were accomplished. The aircraft flew at a height of 300 m and a ground speed of around 70 m/s. Operating with a laser frequency of 5 Hz, we obtained the fluorescence of a sediment surface about 50 cm in diameter at every 14 m. A photo camera was also mounted in the aircraft and took photographs at five second intervals (i.e. 350 m) along the flight track. As shown in table 4, in vivo fluorescence spectra of benthic diatoms in Wadden Sea sediments were measured using both the MiLF and the LFS. An estimation of the pigment in the top 0.5 cm layer of sediments was made once again by taking sediment samples and using the lens tissue technique (Stal et al., 1984, Wonneberger et al., 1984).

Pigment concentrations and vertical nutrient effluxes

The nutrient content in the pore water was analyzed by biologists from the Department of Biology, University of Oldenburg, according to Grasshoff (Grasshoff, 1983; Wang, 1992). The correlation analysis results are shown in table 5, indicating a good correlation between the content of pigments and that of nutrients.

Analysis of in vivo fluorescence obtained with the MiLF

Fig. 13 shows the change of in vivo fluorescence spectra of

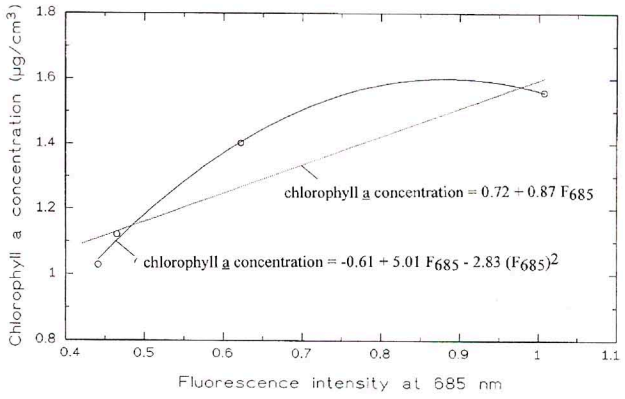

Fig. 13 - Change of the in vivo fluorescence of benthic diatoms with the chlorophyll a concentration in the top 0.5 cm layer of sediments. Circles here represent the measured values. Correction of the chlorophyll a self-absorption was not performed because of the insufficient dynamics of chlorophyll a concentrations.

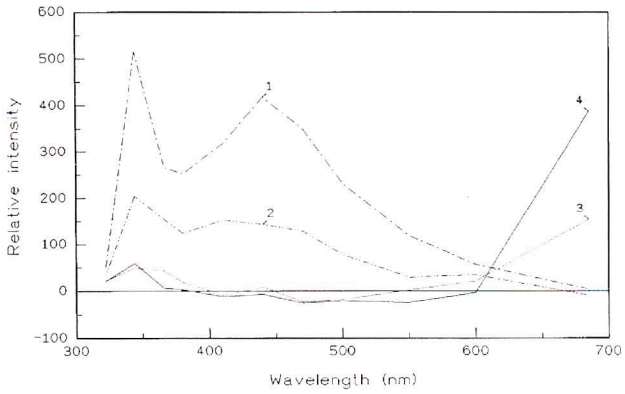

Fig. 14 - Typical fluorescence spectra measured with the LFS during campaign II (λ_{ex} = 308 nm): Water in tidal inlets (1), sediments near tidal inlets with thin water coverages (2), sediments (3) and sediments with a visible film of benthic diatom assemblages (4).

Wadden Sea sediments with the chlorophyll a concentration. The fluorescence at 685 nm shows a high correlation with the chlorophyll a concentration in Wadden Sea sediments. A linear and nonlinear regression of these two parameters gives the following equations (n=4, r=0.91):

$$\text{chlorophyll a concentration} = 0.72 + 0.87\ F_{685} \qquad (8a)$$

$$\text{chlorophyll a concentration} = -0.61 + 5.01\ F_{685} - 2.83\ (F_{685})^2 \qquad (8b)$$

Eq. (8a) is obtained by fitting all four data points with a linear model. According to this equation, chlorophyll a at zero concentration produces negative fluorescence at 685 nm. The nonlinear model in Eq. (8b) shows a good agreement between the theoretical and the experimental data (Fig. 13). This will be further investigated due to the small dynamics of the data obtained during experiments.

Analysis of in vivo fluorescence obtained with the LFS

Typical fluorescence spectra obtained with the LFS are shown in Fig. 14 and are similar to the spectra shown in Fig. 10 with the exception of the water Raman scattering at 366 nm (excitation at 308 nm). Due to the relatively deep water column in tidal inlets, a strong water Raman scattering was superimposed onto the water fluorescence.

Figs. 15 and 16 show typical fluorescence changes along two flight tracks. Gelbstoff fluorescence correlates very strongly with the water Raman scattering, because organic substances in water give rise to much stronger fluorescence than those originating from Wadden Sea sediments. The strong fluorescence at about 685 nm observed on each side of the tidal inlet may be due to a thick film of benthic diatom assemblages which correlated with the nutrient supplies and water content in sediments. It was observed during experiments that there are very dry films of benthic diatom assemblages in the top layers of sediment far from tidal inlets and, in contrast to this, thick films of benthic diatom assemblages which grew on the top layers of humid sediments along tidal inlets.

Based on the data obtained during six flights, maps of chlorophyll a fluorescence at 685 nm and gelbstoff fluorescence at 440 nm were produced by means of interpolation in combination with an algorithm for surface smoothing (Figs. 17 and 18, Wang, 1992). These figures clearly show the negative correlation between chlorophyll a and gelbstoff 308 nm fluorescence, in particular at the positions of tidal inlets I and IV. Tidal inlets II and III are not completely covered due to the shortage of data in some flight tracks and the narrow width of these two tidal inlets (5 Hz laser frequency leads to measurements at 14 m intervals).

Figs. 17 and 18 constitute the basis for an estimate of the phytobenthic biomass in Wadden Sea sediments using the following procedures:

1). Fluorescence measurements and ground truth measurements
This includes fluorescence measurements with a laser fluorosensor and ground measurements by sampling in training areas.

2). Data correction
This step consists of spectral, instrumental, fluorescence self-absorption, second fluorescence corrections and corrections of other nonlinear effects such as the vertical movements of benthic diatoms if required.

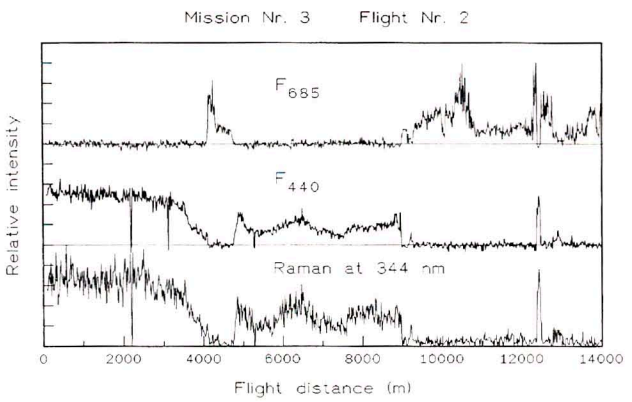

Fig. 15 - Change of fluorescence spectra along flight track 2, which began at a position of 8° 83', 53° 36.0', where an optically deep water column existed. A small dry-fallen tidal flat at a flight distance of about 4.8 km resulted in an abrupt increase of the chlorophyll a fluorescence and the disappearance of the water Raman scattering (8° 64', 53° 37.7'). The water Raman peak at a flight distance of 13 km was due to the water in tidal inlet IV.

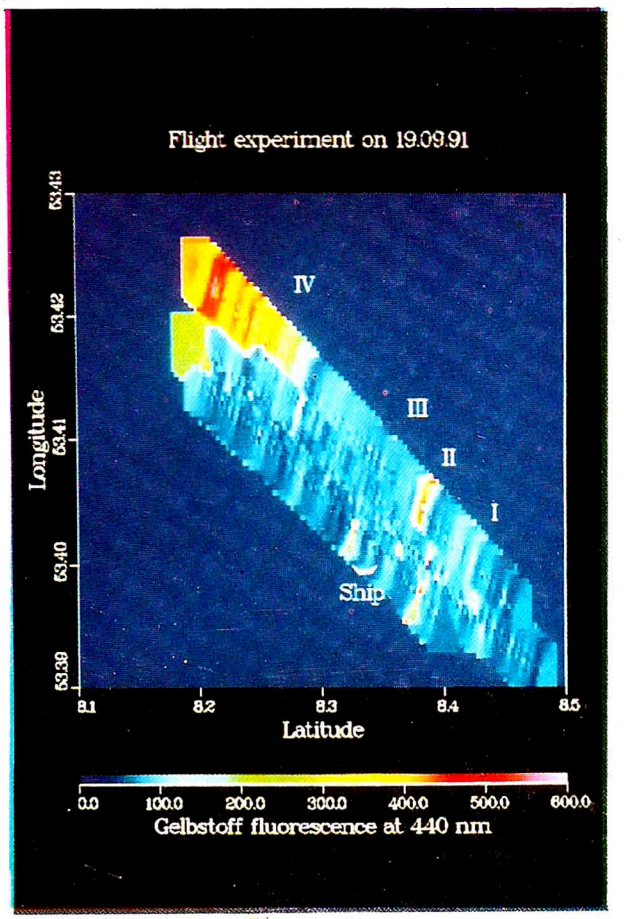

Fig. 17 - Change of gelbstoff fluorescence at 440 nm along the intersections of a total of 6 flight tracks. A blue background is assigned to positions where no data were available. The fluorescence becomes very strong following the direction of the tidal inlets I, II, III and IV.

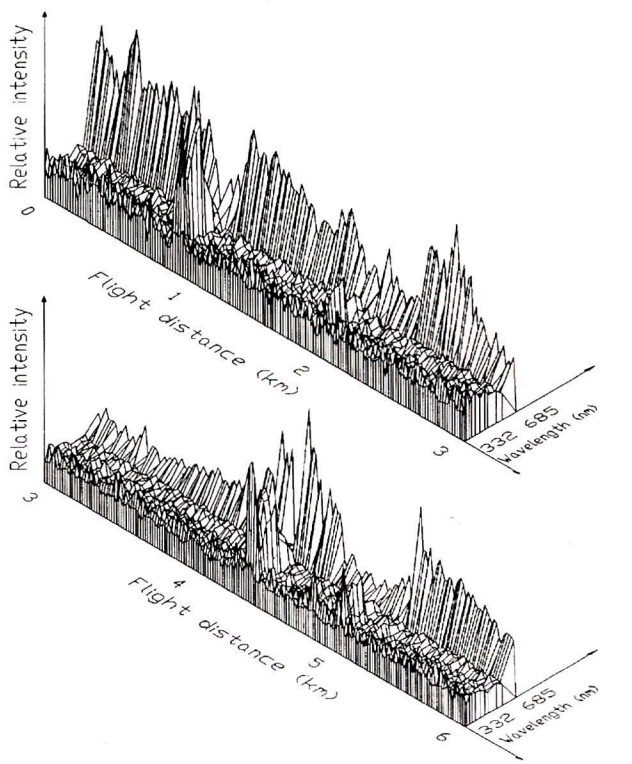

Fig. 16 - Change of fluorescence spectra along flight track 6. The water Raman peaks at flight distances of 1.2, 2.2, 3.5 and 4.6 to 5.5 km were due to the water in tidal inlets I, II, III and IV. The lower part of the figure continues the upper one.

3). Data processing and presentation of results
This key step to interpreting data is carried out with emphasis on the questions addressed at the beginning.

4). Distribution of data to users
Users, like public agencies, need different data combinations for their particular purpose. This last step therefore depends very much on the users requirements and could be very well supported by an efficient programme of monitoring the Wadden Sea.

These steps are interdependent, i.e. information obtained in one step can be used to improve the design of a plan, to support the results of experiments and to perfect the presentation of these results in the other three steps. Passive sensors such as the MSS, and aerial and satellite photography can also be combined in these steps.

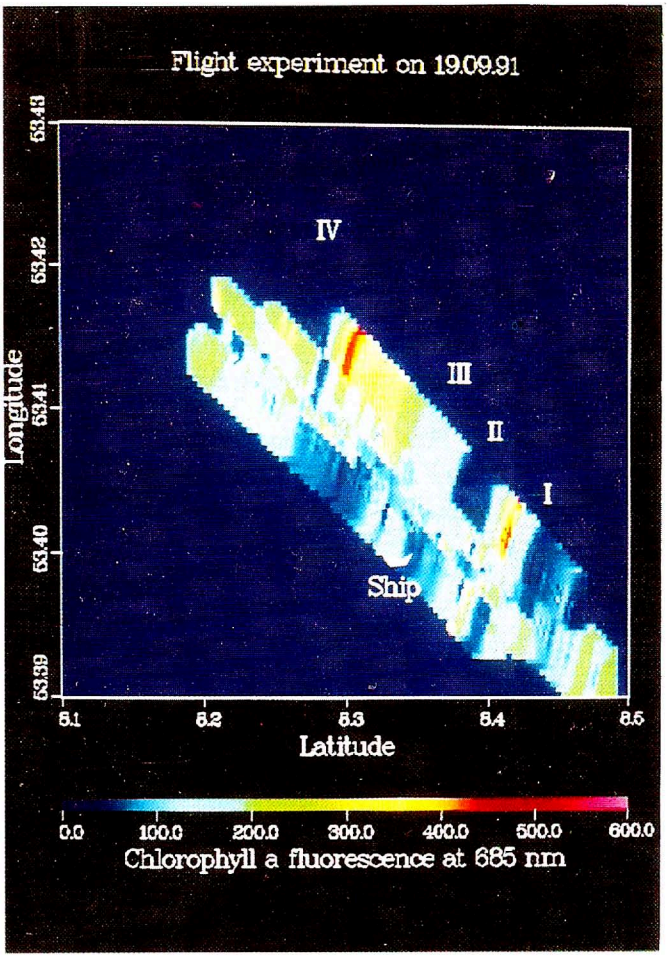

Fig. 18 - Change of chlorophyll a fluorescence at 685 nm along the intersections of a total of 6 flight tracks. The chlorophyll a fluorescence disappears at positions where there are tidal inlets, negatively correlating with the water Raman scattering in comparison to Fig. 17. A blue background is assigned to positions where no data were available.

5. SUMMARY

In summary, it can be concluded that: 1) Gelbstoff and chlorophyll a are two optically dominant constituents of the Wadden Sea which correlate with the measured fluorescence of these substances; 2) This fluorescence can be remotely measured for classifying tidal flats; 3) Correction of this fluorescence results in a more accurate determination of chlorophyll a concentration if fluorescence quenching effects at high chlorophyll a concentrations and the upward movements of benthic diatoms are taken into account; 4) Chlorophyll a fluorescence of the benthic diatoms can be measured remotely using airborne laser fluorosensors and interpreted quantitatively if ground measurements are performed simultaneously.

The fluorescence technique was used for the first time in this investigation to study benthic diatoms and their self-absorption and migration. The laser fluorosensor makes it possible to study the microphytobenthos without touching or disturbing it. It is expected that with the laser fluorosensor an estimate of the biomass can be made which is not absolutely quantitative. However, this information can be used to compare its relative variability in different areas over a long time interval. A quantitative measurement of chlorophyll a fluorescence in Wadden Sea sediments using laser fluorosensors will be more reliable if the following factors are taken into account:

* As described above, corrections to the in vivo chlorophyll a fluorescence should be made in order to obtain a more precise estimate of chlorophyll a concentrations, which in turn give an estimate of the phytobenthic biomass in the Wadden Sea. Ground sampling methods tend to underestimate or overestimate the phytobenthic biomass by a factor of up to 3 (Wolff, 1979). Corrections taking into account the organic carbon make the biomass measurement more accurate (de Jonge, 1980) but also more laborious. Even now, the chlorophyll a content obtained by means of ground sampling without the organic carbon correction is the most popular method for determining biomass. It is therefore suggested that a linear mode is used for laser fluorosensing of chlorophyll a in benthic diatoms in the Wadden Sea, except when the ratio F_{735}/F_{685} is greater than 0.5. In such a case, a nonlinear model should be used to obtain a more accurate estimate of the chlorophyll a concentration from the fluorescence measured using laser fluorosensors.

* As is known, Wadden Sea sediments contain not only chlorophyll a but also gelbstoff and non-fluorescent particles (Laane, 1984). This results in both the absorption and the fluorescence of one component being influenced by the other two co-existing components. An algorithm is therefore needed to quantitatively estimate the chlorophyll a concentration and gelbstoff content of benthic diatoms by means of laser-induced fluorescence and to take into account in more detail the corrections of the fluorescence self-quenching effect, the interactions between two photosystems of chlorophyll a, and the mutual influences among various components in Wadden Sea sediments.

* Due to the thin organic coverage on the top sediment layer, it is suggested that the fluorescence measurement begins half an hour after the Wadden Sea sediments have dried. This allows the benthic diatoms enough time to migrate out of their coverage, to get recovered and to minimize the influence of the upward movements of

benthic diatoms on the measured fluorescence signals. Otherwise, a correction model should be developed on the basis of fluorescence measurements frequently carried out during ebb. Using a similar method, the reasons for a decrease of fluorescence in Wadden Sea sediments after one and half an hours of low water should be examined in more detail, and a related nonlinear correction should also be made.

* Nutrient stresses may result in a change of fluorescence efficiencies in benthic diatoms, a phenomenon which so far has not been studied much (Kiefer, 1973). These stresses may exist in areas where thick benthic diatom assemblages accumulate and sediments exposed to sunlight become too dry during ebb tide.

* The fluorescence efficiency of benthic diatoms can be influenced by continuous light (i.e. sunlight). Referring to the reports by Kiefer (1973) and Günther (1986), it can be expected that a change in chlorophyll a fluorescence efficiency by a factor of up to 2 may be found if benthic diatoms are illuminated with a continuous light source. Simultaneous measurements of the solar irradiance are therefore recommended during experiments with laser remote fluorosensing of the phytobenthic biomass in Wadden Sea sediments. The correction model proposed by Günther (1986) can be used for a more precise assessment of the phytobenthic biomass using chlorophyll a fluorescence at 685 nm if the solar irradiance fluctuates by a factor higher than 2 during experiments.

* Better correlation between the chlorophyll a concentration and the in vivo fluorescence intensity is found when the chlorophyll a concentration in the thin upper layer of sediment is determined. This is understandable, because the in vivo fluorescence originates in the upper surface of sediment layers. The thinner the sediment layers sampled, the more chlorophyll a is correlated with the fluorescence signal, because the upward movement of benthic diatoms results in accumulations of benthic diatoms on the Wadden Sea sediment surface. In contrast, pheophytin a is found to increase very quickly with the sampling depth of sediment layers. It is therefore recommended that the thin upper layer of sediment should be sampled for ground measurements and for calibrating the laser fluorosensor.

* In order to get the largest dynamic range of chlorophyll a concentrations, samples should be taken from the places consisting of an area with a thick film of benthic diatom assemblages and a background area, and also the areas between these two. There should be fewer benthic diatoms, and therefore less chlorophyll a, in the background areas.

* More quantitative estimates of phytobenthic biomass and gelbstoff content can be made using fluorescence intensities and in particular, using the three fluorescence parameters described above and their combinations, such as F_{685}/F_{735}, F_{685}/F_{450} and F_{735}/F_{450}, because such parameters have been applied in classifying the Wadden Sea sediments with thin water coverage. Apart from this, the water Raman scattering of Wadden Sea water in tidal inlets should be taken into account if the LFS is used for airborne measurements, because this scattering provides a standard for the gelbstoff fluorescence of Wadden Sea water and sediments (Bristow et al., 1981).

* A nitrogen and an excimer laser have been used in this investigation for chlorophyll a measurements. Using a light beam as an excitation source with a wavelength inside the absorption bands of chlorophyll a, we can reduce the fluorescence disturbances of other fluorescent pigments and organic substances in assessing the phytobenthic biomass in Wadden Sea sediments. In addition to this, pigments in the Wadden Sea other than chlorophyll a can be revealed by changing the excitation wavelength (Yentsch et al., 1979). For example, fluorescence can be measured at 680 nm for chlorophylls when it is excited at 450 nm; and for fucoxanthin when excited between 560 and 570 nm. The fluorescence of phycoerythrin can be detected in a range from 560 to 570 nm with an excitation wavelength of 490 nm. It is expected that these non-chlorophyll a pigments can also be used as parameters for remote sensing of the Wadden Sea, as for the open sea (Hoge et al., 1986).

Finally, it is expected that the information presented in this paper will be helpful in giving strong backing to the establishing of a long-term, large-area programme of monitoring the Wadden Sea ecosystem in future by means of a laser fluorosensor, particularly with regard to the continuous operation of the new sensor package mentioned in the introduction. This programme includes significant efforts directed to the sensor calibration and data validation, collection, processing, archiving and distribution. Such a programme could establish the current status of the Wadden Sea and assess the extent of possible problems. However, it must be continued for many years before any statistically defensible approach can reveal the trends.

The LFS provides only one of many methods for monitoring the Wadden Sea, and its combination with other methods is necessary and also beneficial. This will enhance the LFS' functions and make it more efficient.

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