NASA TECHNICAL MEMORANDUM

NASA TM - 76626

LASER INDUCED FLUORESCENCE FROM ALGAE - RESULTS OF A SHIP-BORNE FIELD TEST

Britt Hartmann, Ove Steinvall, Anders Widén

NASA-TM-76626 19820003885

Translation of "Laserinducerad fluorescens fraan alger - Resultat fraan ett baatburet faeltfoersoek", Research Institute of National Defence, Stockholm (Sweden). Report No. FOA-C-30171-E1, July 1979, pp. 1-51

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 SEPTEMBER 1981

STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
NASA TM-76626		

4. Title and Subtitle	5. Report Date
LASER INDUCED FLUOROESCENCE FROM ALGAE - RESULTS OF A SHIP-BORNE FIELD TEST	September 1981
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Britt Hartmann, Ove Steinvall, Anders Widén	
	10. Work Unit No.

9. Performing Organization Name and Address	11. Contract or Grant No.
Leo Kanner Associates Redwood City, California 94063-0187	NASW-3541
	13. Type of Report and Period Covered
	Translation
12. Sponsoring Agency Name and Address	14. Sponsoring Agency Code
National Aeronautics and Space Administration Washington, D.C. 20546	

15. Supplementary Notes

Translation of "Laserinducerad fluorescens fraan alger - Resultat fraan ett baatburet faeltfoersoek", Research Institute of National Defence, Stockholm (Sweden). Report No. FOA-C-30171-E1, July 1979, pp. 1-51. (N80-18678)

16. Abstract

The report concerns laser induced fluorescence from algae during a ship-borne field test in the Baltic Sea. It aimed at providing a basic for the feasibility of air-borne laser fluorosensing not only of chlorophyll but also of pollutants such as oils and chemicals. There was a satisfactory correlation between the laser data and those obtained manually. Some uncertainty concerns the absolute determination of the chlorophyll concentration. Airborne tests should be complemented with "sea-truth" measurements within the area surveyed. The work was performed in cooperation between the Zoological Institute of Stockholm University and the Swedish Nature Concervancy Board.

17. Key Words (Selected by Author(s))	18. Distribution Statement
	Unclassified - Unlimited

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22.
Unclassified	Unclassified	42	

NASA-HQ

N82-11758#

LASER INDUCED FLUORESCENCE FROM ALGAE - RESULTS OF A SHIP-BORNE FIELD TEST

Britt Hartmann, Ove Steinvall, Anders Widén
The Armed Forces Research Institute, Main Dept. 3,
581 11 Linkoeping, Sweden

Abstract

The report furnishes an account of measurements of laser induced /1*
fluorescence from algae during a ship-borne experiment in the Baltic Sea. It aimed at providing a basis for evaluating the feasibility of an air-borne laser fluorosensor to be used primarily for surveying chlorophyll but also for identifying water pollutants in the form of oils and chemicals. The results indicate a close agreement between the laser data and the results obtained by manual methods. However, there is a slight uncertainty during absolute determination of the chlorophyll concentration, indicating that the system of air-borne remote sensing laser analysis should be complemented with "sea-truth" measurements at some points within the area surveyed.

This work was performed in cooperation with the Zoological Institute of Stockholm University and the National Swedish Nature Conservancy Board.

Order no.: ER 09C

Distribution list: FMV-A, FMV-M, FMV-F, CTH, KTH, LTH, LITH, UU Teknicum, Sjoev. Havd. 1, Havd 2, Havd 4, Havd 5.
Havd. 3: 300, 330, 333, 345, 348, 360, 390, 314.

---

* Numbers in the margin indicate pagination in the foreign text.

CONTENTS

		Page orig.	Page transl.
	Abstract	1	1
1.	INTRODUCTION	3	3
2.	LASER INDUCED FLUORESCENCE FROM ALGAE LABORATORY TESTS	4	4
3.	DESCRIPTION OF FIELD TEST INSTRUMENTATION	11	9
4.	EXPERIMENTS PERFORMED	13	11
4.1	Preliminary Tests	13	11
4.2	Field Tests	18	11
4.2.1	Installation of the Laser System	18	15
4.2.2	Test Program	20	15
4.2.3	Test Results	22	18
	o Presentation of the Results	22	18
	o Results June 13, 1978	26	22
	o Results June 14, 1978	31	24
	o Results June 15, 1978	31	27
	o Numerical evaluation of the fluorescence profile of the measuring data	43	34
5.	CONCLUSION	46	37
	REFERENCES	48	39
	APPENDICES	49	40

1. INTRODUCTION /3

The increased need for knowledge of water quality and the productive potential of lakes, coastal areas and seas for the utilization of these areas as resources for recreation, fishing or as locales of industries has led to an increased collection of data concerning status descriptions, documentation and surveyance of these areas.

In the use of remote sensing analytical methods when collecting the relevant data, we have at our disposal a sampling system with a wide active radius and a speed of sampling allowing us to test several measuring points and areas within a short timespan.

One such parameter is the amount of chlorophyll in open waters, reflecting the biomass of the phytoplanktonic algae and their species composition, which are in turn governed by physical/chemical conditions such as access to nutrient salts, etc. The activity of the chlorophyll-containing phytoplanktonic algae when solar energy is absorbed and converted into energy-rich chemical compounds, reducing carbon dioxide to carbohydrates, forms the basis ecologically for the fish production and, consequently, for the economical return of the catch.

Mapping the distribution of the algae will also furnish information on the productive capacity of aqueous areas as well as provide indirect information on any possible outlets of pollutants, the conditions of currents, etc.

By the introduction of remote sensing analyses into aquatic research, methods have become available for recording large scale distribution of chlorophyll in a water body. For this purpose, a passive system of multispectral scanner type has been used operated either from an airplane or from a satellite.

The advantage of an active system, for instance a laser fluorosensor, consists in that it can be used around the clock and in that the return signal corresponds specifically to the amount of living algae, in principle able to be calibrated against an absolute scale. The possibility for varying the wavelengths emitted by a tunable laser increases the probability of detection and identification since different classes of algae posses different fluorescence and excitation spectra. /4

The present report gives an account of the composition of a laser system for, among others, fluorescence measurements and contains the results of some

preliminary ship-borne field tests. The work was in part performed as a civilian commitment by the Zoological Institute of Stockholm University, where Bo Nyquist functioned as contact man.

2. LASER INDUCED FLUORESCENCE FROM ALGAE - LABORATORY TESTS

The light absorption of plants takes place in the pigment molecule. There are different kinds of color pigments of which chlorophyll is one. Each has a characteristic absorption spectrum within the ultraviolet and visible ranges. For instance, chlorophyll absorbs within the red and the blue, resulting in the green color of the plants.

The absorbed energy can be utilized either for the photosynthetic process or be emitted as fluorescent radiation. The algae have species-related sets of pigments, leading to typical absorption and fluorescence spectra. A fluorescence peak at 685 nm, originating from chlorophyll a, is characteristic of all living algae. Other pigments frequently transmit their absorbed energy to the cholorphyll before they themselves have time to fluoresce.

The algae can be divided into three main groups on the basis of their /5
pigment composition: the bluegreen, the green and the red as well as the diatoms (armored flagellates). It has been demonstrated that within each of the main taxa [1] the excitation and emission spectra are fairly uniform. If the spectrum and the profile of the process in question are known, it is in principle possible to determine the concentration of the different groups of algae by means of a remote sensing laser system.

As preparation for the field tests using a remote sensing laser system, we did laboratory experiments on the fluorescence of a large number of algal samples, using the equipment illustrated in Figure 1.

A tunable dye laser with emission within both the UV and the visible portions of the spectrum excited suspensions of algae. The fluorescent light was collected by a lens and analyzed by means of a lattice monochromator, provided with a photomultiplier.

The pulses from the photomultiplier were averaged in a box car integrator and the signal thus obtained together with a voltage proportional to the rotation of the lattice was conducted to an X-Y recorder, presenting the fluorescence spectrum.

LAB-FÖRSÖK. 1

/6

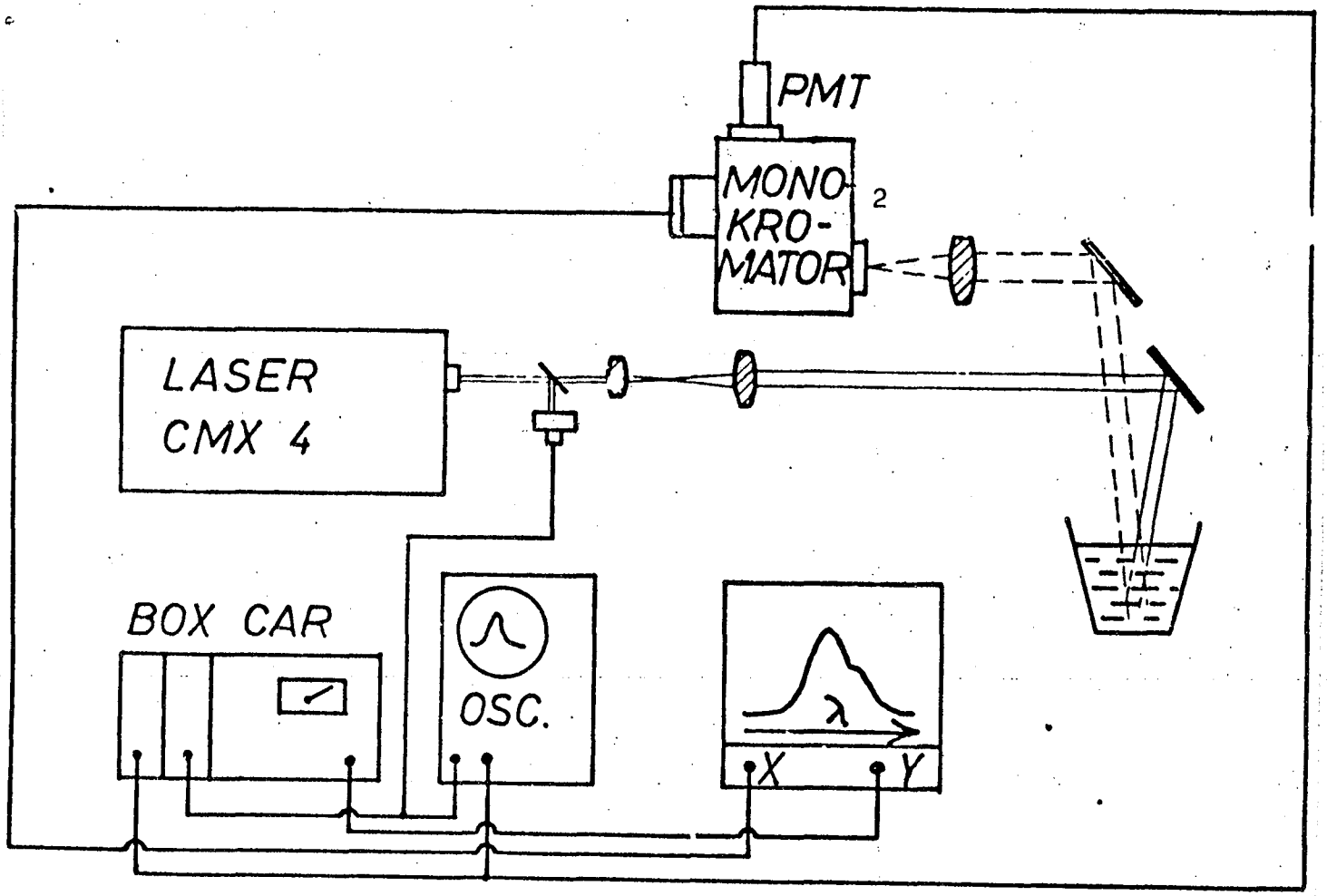


Figure 1. Laser instrumentation for a laboratory test of laser induced fluorescence. Key: 1. Lab test, 2. monochromator.

Figure 2 shows examples of some fluorescence spectra at an excitation wavelength /7 of 600 nm. The signal measured was calibrated against the fluorescent intensity of rhodamine 6 G, measured in an identical manner. The tests revealed that the chlorophyll a in algae has a fluorescence maximum within the range of 683-688 nm with a half-width of 20 nm. Bluegreen algae have in addition a fluorescence maximum at 660 m. The fluorescent intensity of different kinds of algae varied not only due to the excitatory wavelength but also due to the physiological status, i.e.

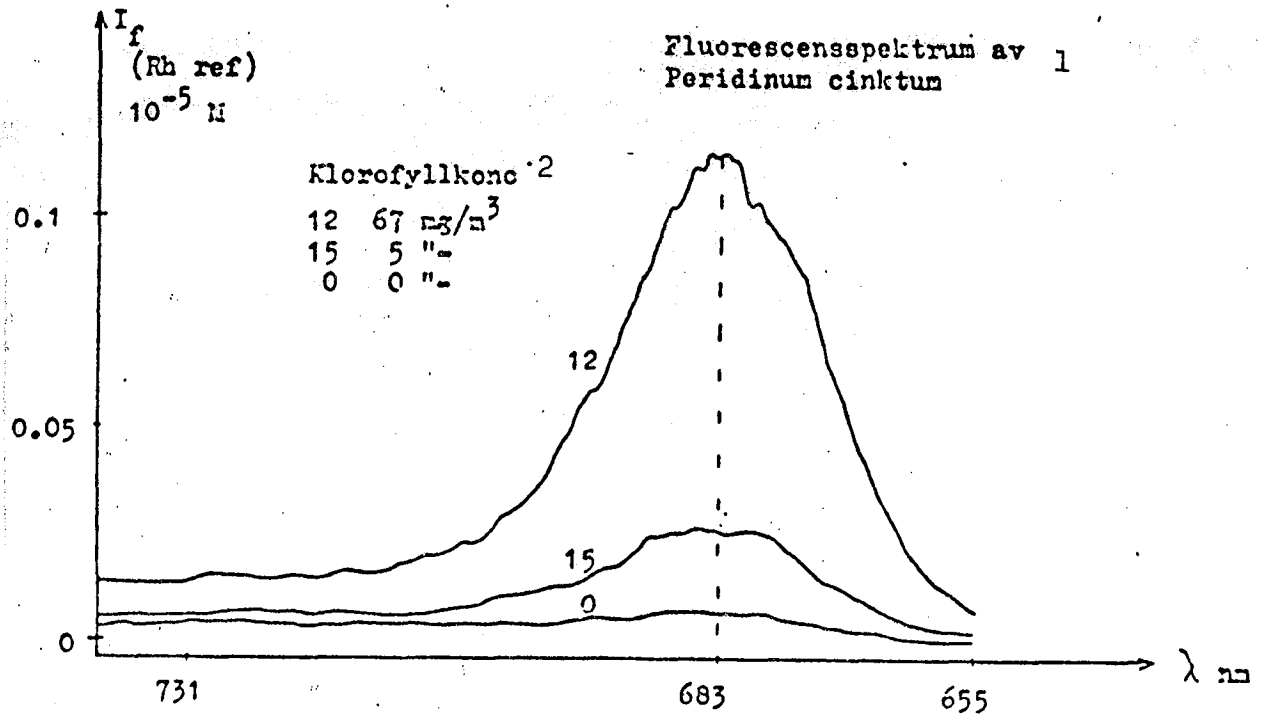


Fig. 2 a. λ_{ex} 490 nm, $\Delta\lambda_f$ 2 nm, p_l 0.8 kW

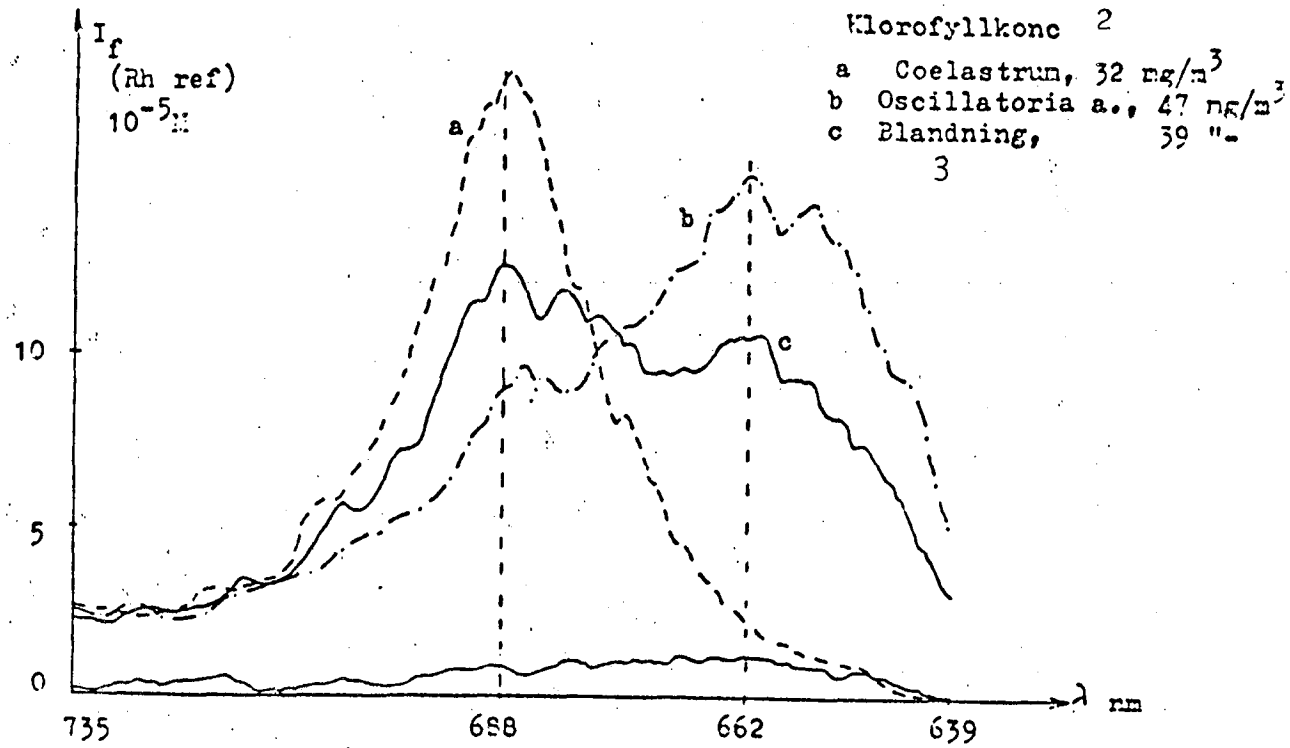
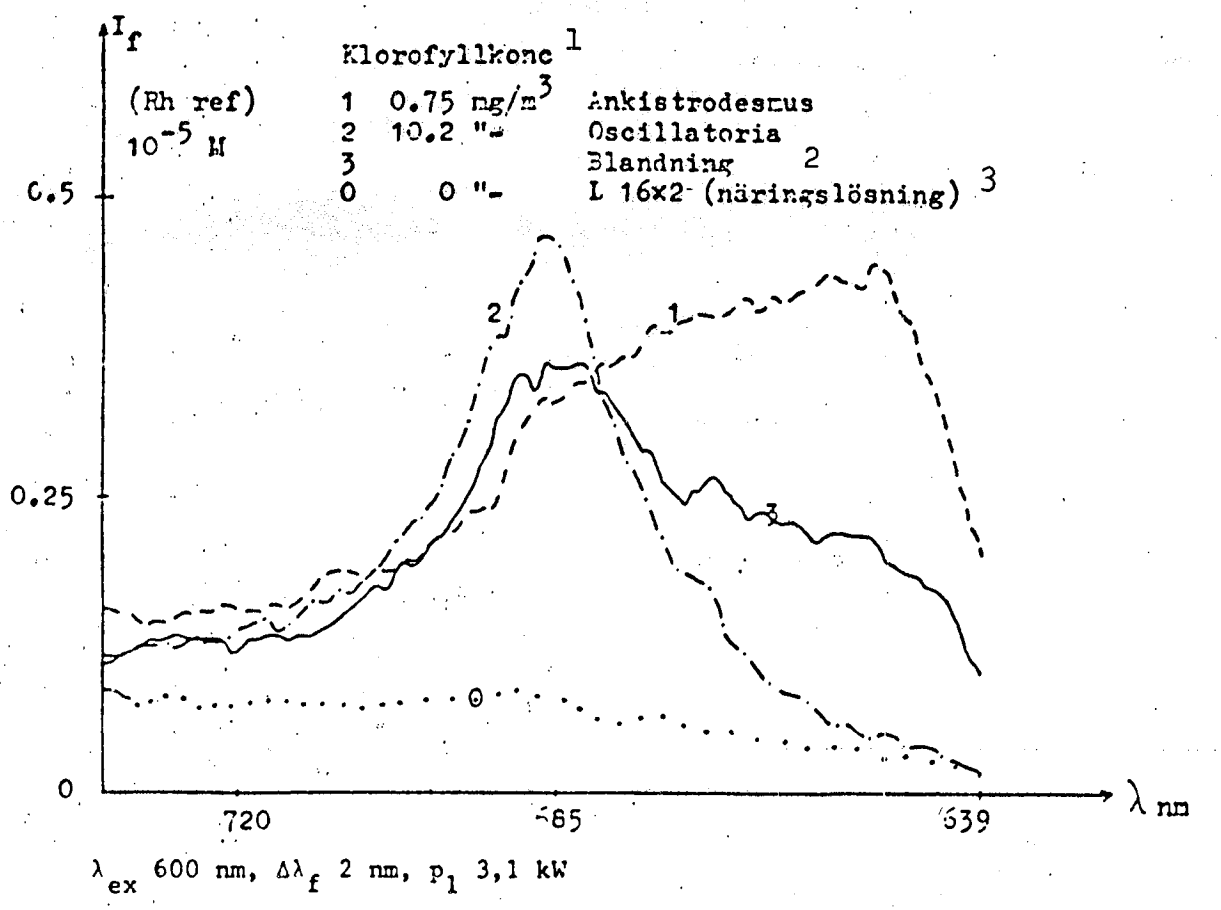


Fig. 2 b. λ_{ex} 500 nm, $\Delta\lambda$ 2 nm, p_l 3.1 kW

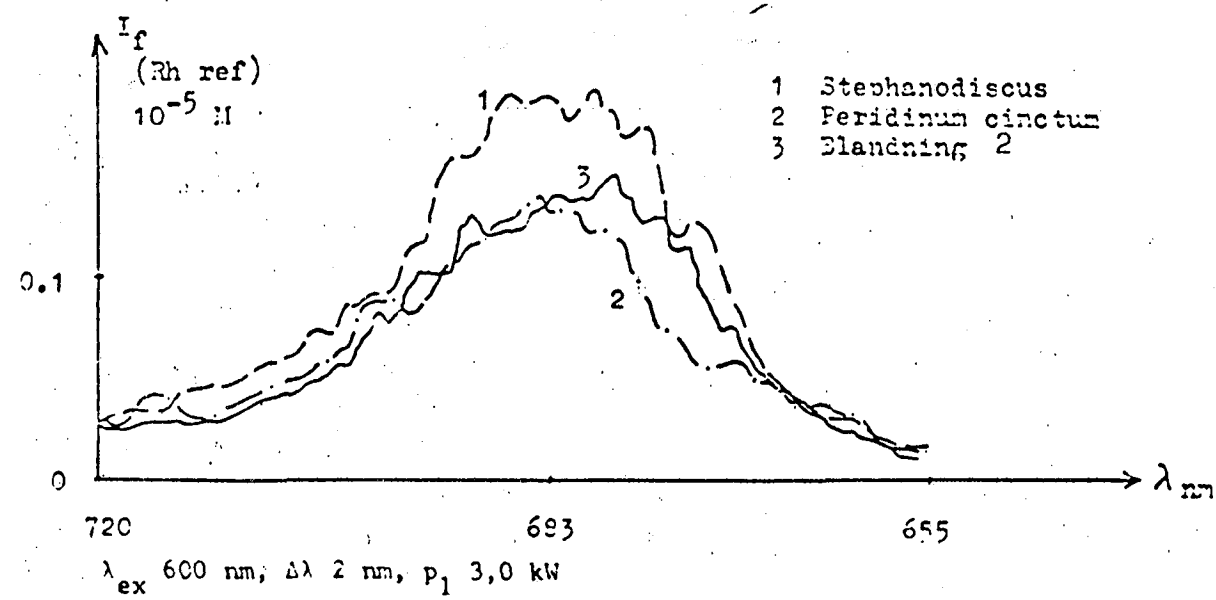
Figure 2 a-b. Some examples of fluorescence spectra measured from laser excited algal suspensions. Volume of liquid: 0.5 liter.

Key: 1. Fluorescence spectra of Peridinium cinctum
2. Chlorophyll concentration
3. Mixture



λ_{ex} 600 nm, $\Delta\lambda_f$ 2 nm, p_l 3,1 kW

Figur 2 c.

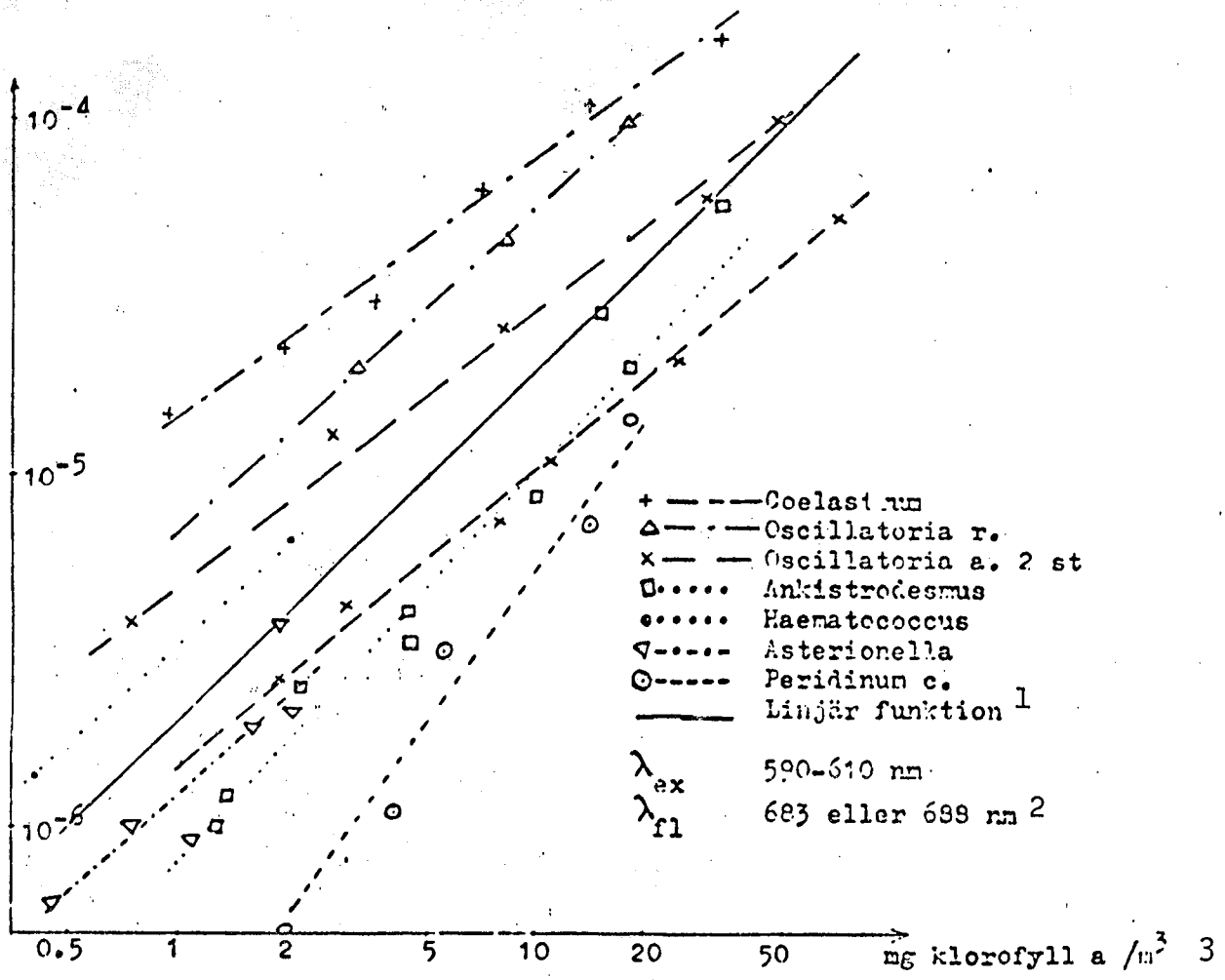


λ_{ex} 600 nm, $\Delta\lambda$ 2 nm, p_l 3,0 kW

Figur 2 d.

Figure 2 c-d. Some examples of fluorescence spectra measured from laser excited algal suspensions. Volume of liquid: 0.5 liter.

Key: 1. Chlorophyll concentration
2. Mixture
3. Nutrient solution

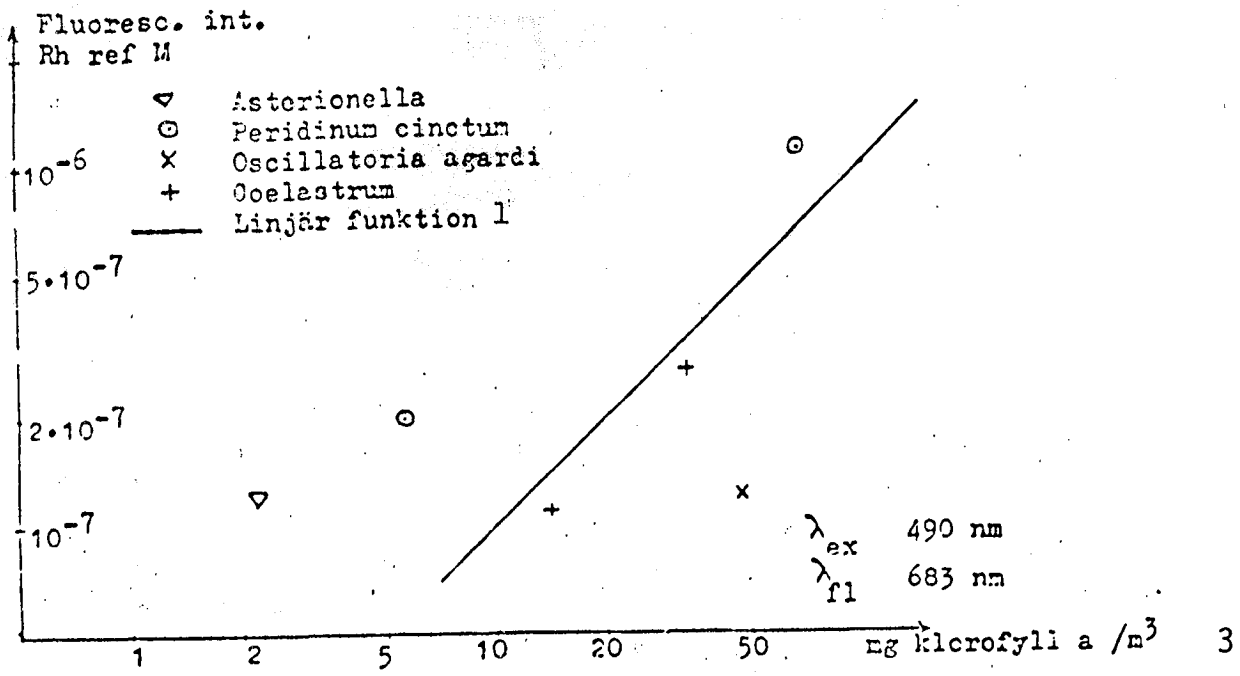


/10

Figure 3. (see text). Key: 1. Linear function; 2. 683 or 688 nm, 3. chlorophyll a / m3.

Figures 3 and 4 demonstrate fluorescent emissions from various kinds of algae /7 in relation to corresponding fluorescence from rhodamine 6 G. In most cases, a close linear relationship was noted between the intensity of the fluorescence and the concentration of chlorophyll a within the range of 0.5 - 10 µg/ℓ (the volume of the liquid was 0.5 ℓ). In contrast, the intensity of the fluorescence differs by more than a factor of 10 between the weakest and the strongest of the signals measured at the same excitatory wavelength. In general a wavelength around 600 nm was more effective for excitation than shorter wavelengths within the visible or ultraviolet ranges.

These preliminary lab tests conducted as a degree project were described in detail in [2]. In [3], similar measurements are reported.



/10

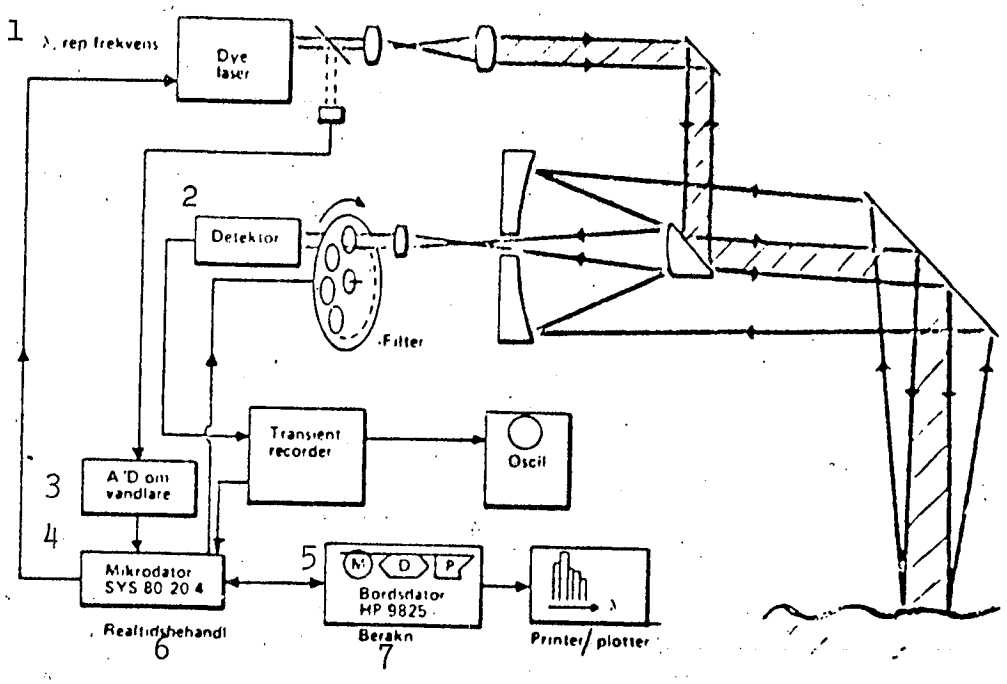
Figure 4. Key: 1. Linear function, 2. chlorophyll a /m^3.

3. DESCRIPTION OF THE FIELD TEST INSTRUMENTATION /11

The instrumentation used for the field tests is sketched in Figure 5. The beam from the flash-bulb pulsed dye laser was expanded to a diameter of about 3 cm before being reflected down onto the water. The pulse effect emitted could be sensed by means of a beam separator in front of the laser and by a photodiode. The fluorescent light from the laser-illuminated water surface was collected by mirror optics with an aperture of 20 cm.

By means of a diaphragm, the view field could be adjusted to a suitable /12 value. After passing an interference filter, the fluorescent light was focused on a Varian VPH-159A photomultiplier with a fairly straight response gradient within the wavelength range of 300-800 nm. The interference filters were placed on a motor-driven wheel with 10 positions. This allowed the fluorescence spectrum to be measured at 10 optional wavelength bands, defined by the interference filters. Data concerning the filters used can be found in Appendix 1.

The portion analyzing the signal consisted of a Biomation 8100 transient recorder, an INTEL SYS 80/20-4 microcomputer, and a HP 9825 desk calculator together with a printer/plotter. This measuring system, which can in principle be considered a general system for testing pulsating fast transients, was developed



/10

Figure 5. LIDAR system for studying, among others, algal fluorescence.

Key: 1. λ rep. frequency
2. detector
3. A/D converter
4. microcomputer
5. desk calculator
6. absolute time treatment
7. calculations

by Gunnar Gustavsson and Olov Lundén at FOA 3 [1]. /12

The analog signal from the photomultiplier is digitalized by the transient recorder into 2048 8-byte words at a sampling rate of 100 MHz. The data are continuously read to the microcomputer which adds them up. Due to the relative slowness of the microcomputer only 256 of the 2048 samples available can be read at the repeater frequency of 25 Hz. By altering the sampling frequencies to below the maximal 100 MHz, the total sampling time can be changed from 2.5 μs and up. This is, however, done at the cost of the time resolution.

The signal formed by the mean of an optional number of pulses is then /13 transferred to the calculator for possible storage on tape or plotting, manipulating, etc. The microcomputer is governed by the calculator via a 15-byte parallel interface. The calculator functions in addition as terminal of the microcomputer, the program of which is usually stored in EFROM, but can also be run in RAM,

---

[1] Swedish Armed Forces Research Institute.

enabling us to make changes in the program. The computer compensates automatically for variations of the background level (background light) on the condition that a calibration is made at the start of the data collection. For each laser pulse, a value of the pulse peak is sampled by a fast A/D converter built into the computer.

4. EXPERIMENTS PERFORMED

4.1 Preliminary Tests

In order to test the field experiment equipment, some preliminary laboratory tests were performed. After a course of 8 m, a laser beam was reflected down into a beaker with a test suspension and the fluorescent signal was measured using different interference filters in comparison with the signal from a 10^{-5} rhodamine 6 g solution. To supress the direct laser reflex, a color filter of type RG-5 was used in addition to the interference filter. It transmits $< 10^{-5}$ at λ = 600 nm and 88% at 685 nm.

Figures 6 and 7 illustrate the fluorescence spectra of some samples of /14
algae. As can be seen, two bluegreen algae (Oscillatoria) have a maximum between 660 and 670 nm in agreement with previous laboratory tests, using analog recording [2]. Figure 7 shows the spectrum of a diatomaceous alga, Stephanodiscus. It has a much weaker spectrum than the green or the bluegreen algae.

Figure 8 demonstrates the fluorescent intensity at 685 nm in comparison with that of 10^{-5} M rhodamine 6 G. These measurements show satisfactory agreement with the corresponding results in [3].

Excitation was also caused by UV light, λ = 300 nm. Table I shows the relationship between the fluorescence return at the laser wavelengths 600 nm and 300 nm (standardizing for laser effect was done).

Excitation at 600 nm is thus more effective that at 300 nm.

4.2 Field Tests

The field tests were conducted on board one of the vessels of the Navy, /18
the "Urd", under the command of FMV-M[2], but usually loaned to the FOA[3] for

---

[2] ? Armed Forces Marine Corps; [3] Armed Forces Research Institute.

TABLE I

/14

Relationship q measured on fluorescence return from some algae, excited at 600 nm and 300 nm wavelengths, respectively.

Kind of algae	q
Osc. Agardi (bluegreen)	180
Oscillatoria (bluegreen)	65
Chlorella (green)	1.7
Stephanodiscus (diatom)	7.2

/15

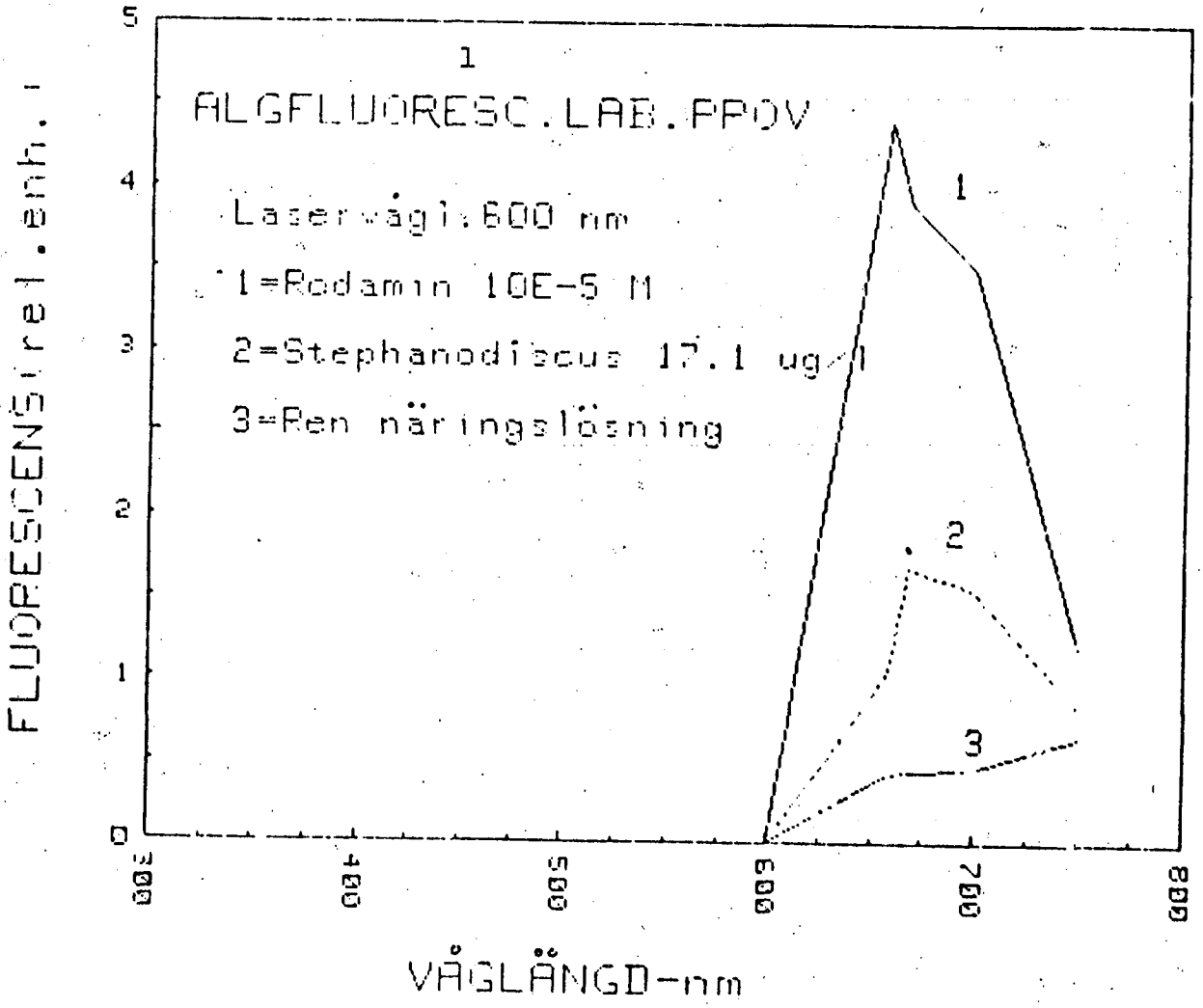
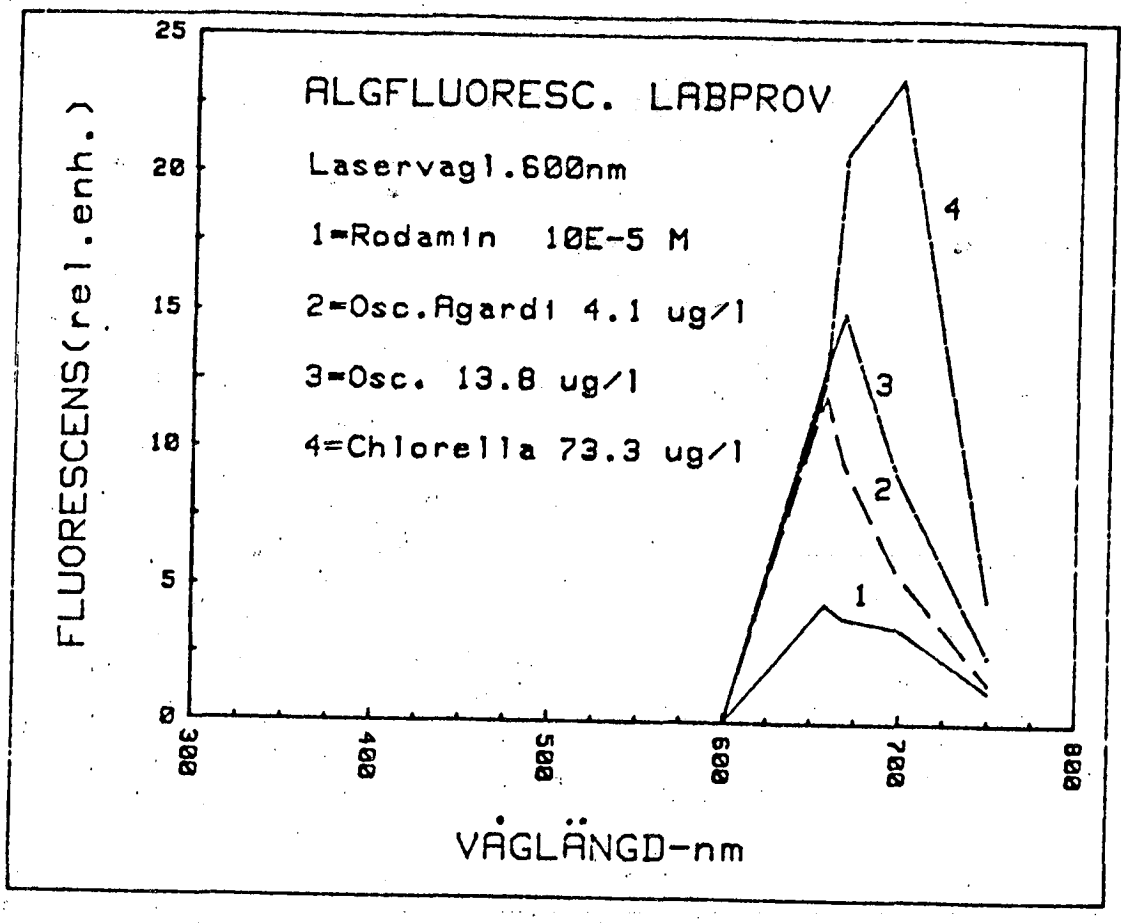


Figure 6. Examples of fluorescence spectra measured in the laboratory, using the same equipment as for the field tests.

Key: 1. Algal fluorescence, Lab test. Laser wavelength 600 nm.
1. Rhodamine 10^{-5} M, 2. Stephanodiscus 17.1 µg/ℓ,
3. pure nutrient solution
2. Fluorescence, rel. units.
3. Wavelength - nm

1



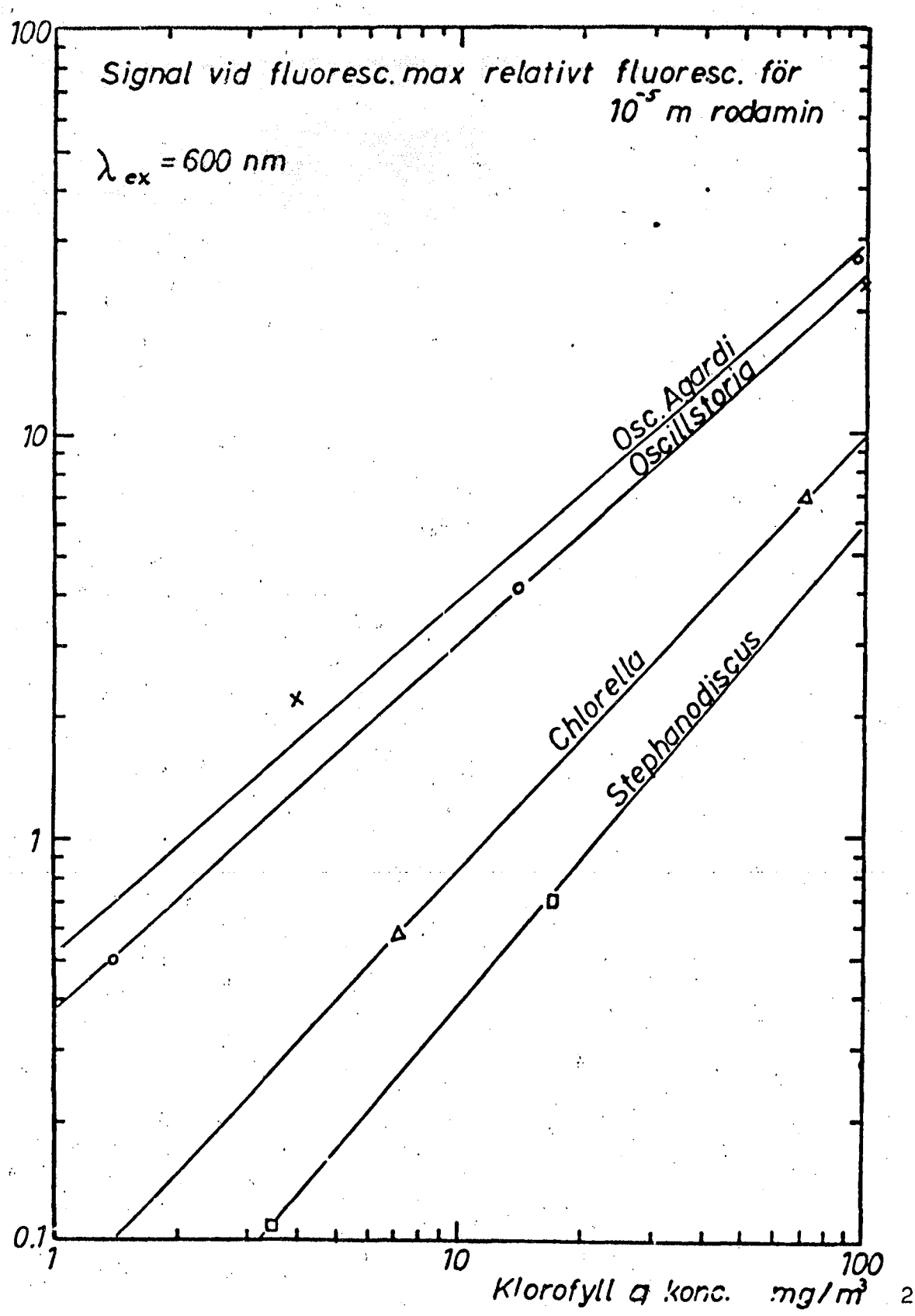
/16

2

3

Figure 7. Examples of fluorescence spectra measured in the laboratory, using the same equipment as for the field tests.

Key: 1. Algal fluorescence. Laboratory test.
Laser wavelength 600 nm
1. Rhodamine 10^{-5} M
2. Osc. Agardi, 4.1 µg/ℓ
3. Osc., 18.8 µg/ℓ
4. Chlorella 73.3 µg/ℓ
2. Fluorescence (rel. units)
3. Wavelength - nm.



1 /17

2

Figure 8. Fluorescent intensity measured on some kinds of algae.

Key: 1. Signal at fluorescent maximum in relation to the fluorescence of rhodamine 10^{-5} M.
2. Chlorophyll a concentration, mg/m^3

experiments. Bo Nyquist from the Askoe Laboratory (of the Zoological Institute, Stockholm University) was the project leader. Together with Bruno Bjoernborg and OEsten Lindgren from the Swedish Nature Conservancy Board (SNV), he performed water analyses,concerning, among others, temperature, conductivity, turbidity, opacity depth and fluorescence as well as taking water samples for later analyses of pigment content and algal composition. The experiments consisted of measurements, conducted over 3 days in the archipelago between Berga near Stockholm and Askoe outside the city of Trosa. /18

4.2.1 Installation of the Laser System

The laser and the accompanying equipment were installed in a relatively large research laboratory below deck. The electrical system of the ship, 220 V single phase 10 A, would have been sufficient for the laser equipment but in order to avoid overloading it, the laser was run by a small, gasoline-powered, electric generator.

The laser beam was emitted along the optical axis of the receiver and angled down onto the water surface by means of three flat mirrors with surfaces somewhat larger than that of the receiving equipment. The distance between the receiver and the surface of the water was 7 m (Figure 9), and the surface of the laser spot on the water surface ca. 3 cm. The spot sensed by the reciever measured ca. 14 cm in diameter. Unfortunately, it was impossible to use this system when cruising due to the formation of foam on the laser-illuminated water surface. Therefore a number of measurements were made in a recess (Figure 9, beam course A' B' C'), where the water surface did not foam in spite of strong agitation of the water volume.

4.2.2 Test Program /20

The test route, the course of which within the archipelago between Berga and Askoe can be seen in Figure 10, was covered in about three days.

The laser measurements were conducted according to the following time schedule:

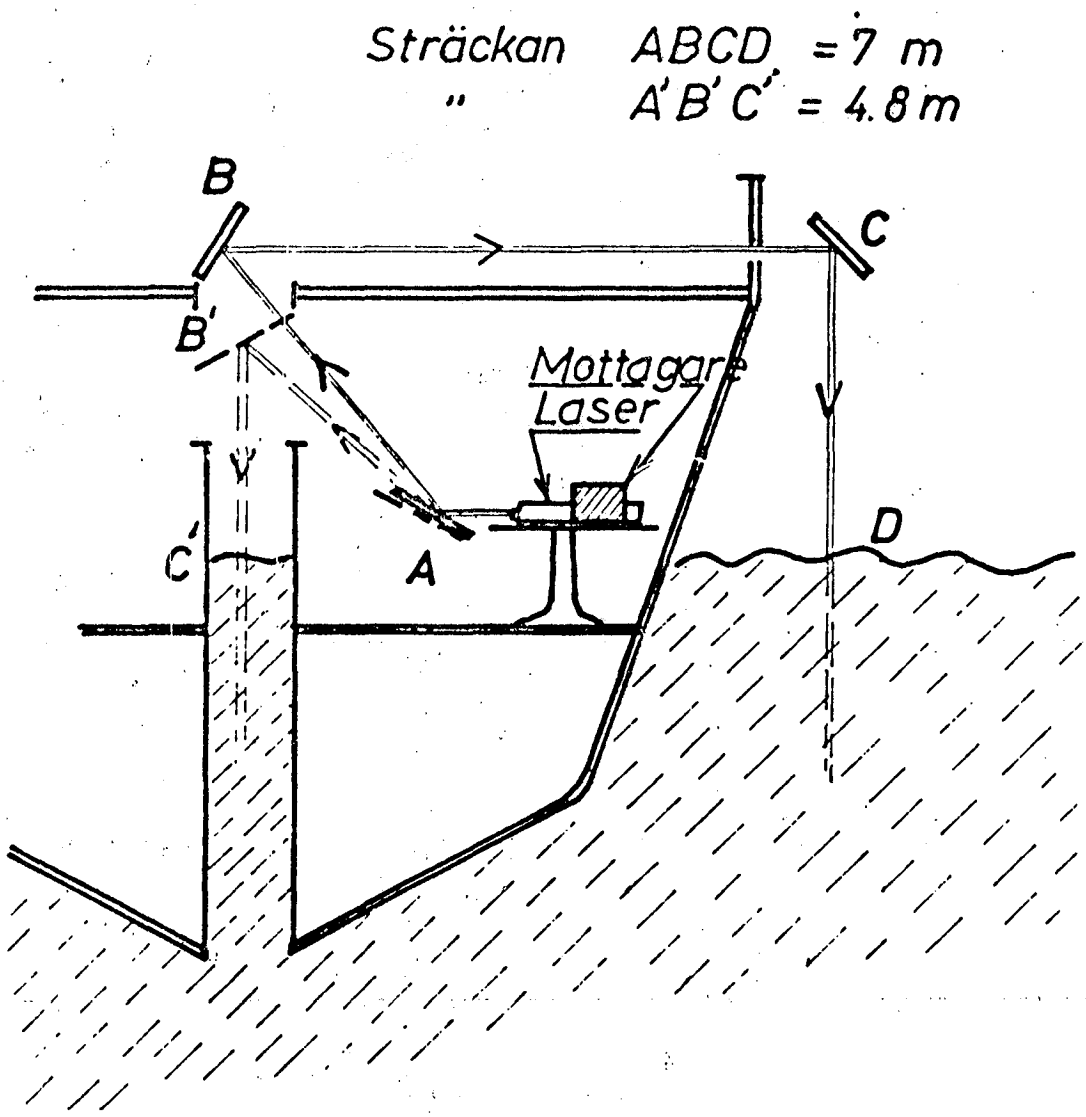


Figure 9. Sketch of part of the test arrangement. Measurements in the recess (A B' C') were made during the last of the three days of testing.

Key: 1. Distance A B C D = 7 m
" A B' C' = 4.8 m

2. Receiver

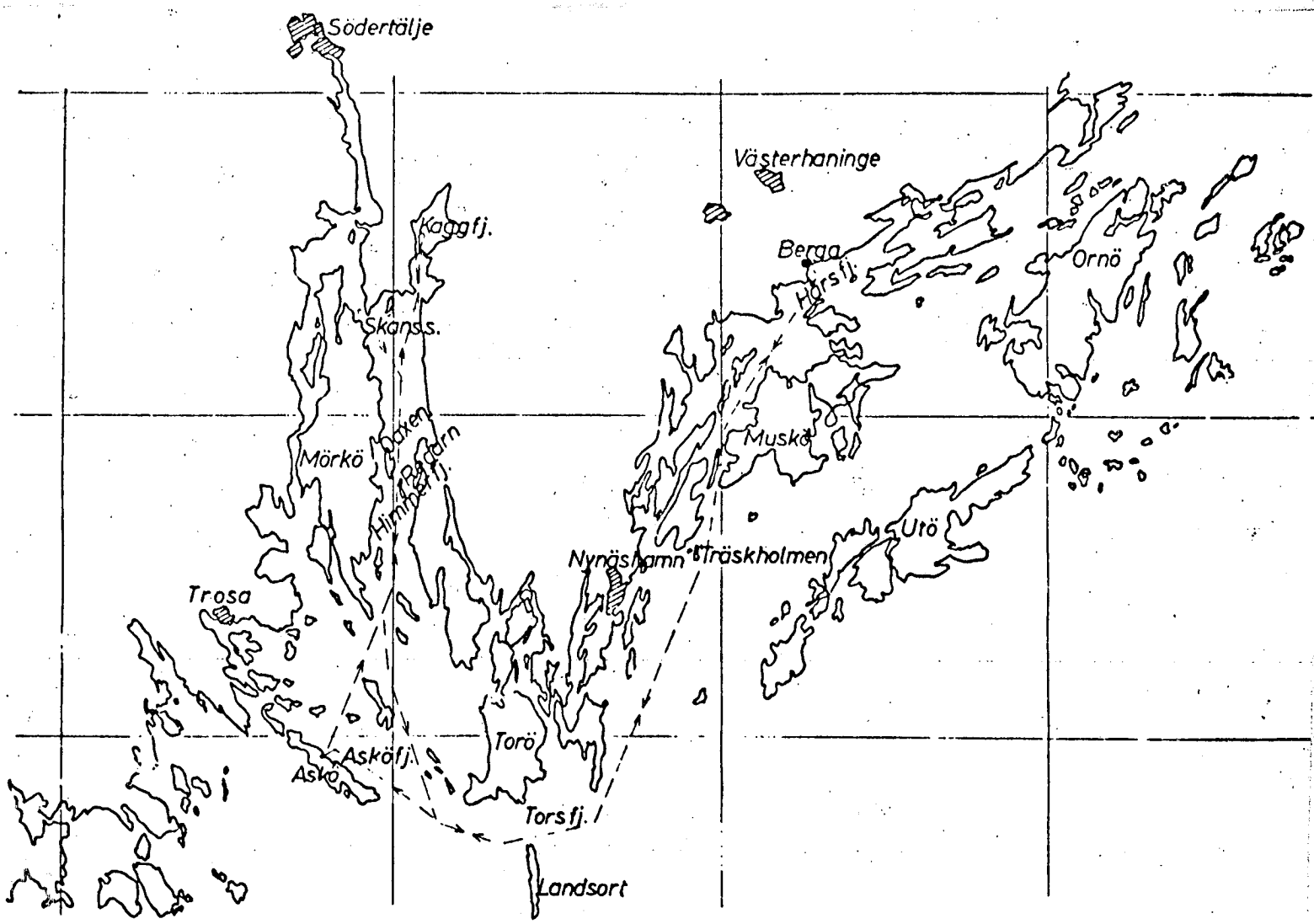


Figure 10. Map of the test route.

Date	Time	Remarks
June 12, 1978	3.45 - 5.00 pm	Preliminary test, Haarsfjaerden, λ_{ex} = 600 nm
June 13, 1978	10.20 am - 1.30 pm	Start from Berga
	3.10 pm - 5.30 pm	Finish at Askoe
June 14, 1978	8.40 am - 10.10 am	Start from Askoe
	12.40 pm - 1.10 pm.	Stop at Oaxen
	2.20 pm - 3.10 pm.	Stop at AEngsholmen
	4.00 pm - 6.30 pm	In Himmersfjaerden - Kaggfjaerden
	8.00 pm - 11.00 pm	Stop in Kaggfjaerden
June 14-15, 1978	11.00 pm - 2.30 am	λ_{ex} = 300 nm; test also for oils in Kaggfjaerden
June 15, 1988	3.40 am - 4.20 am.	λ_{ex} = 490 nm, in Kaggfjaerden
	7.45 am - 7.40 am	
	8.25 am - 10.50 am	Measurements in the recess when
	1.30 pm - 2.40 pm	cruising, λ_{ex} = 600 nm.

/20

4.2.3 Test Results

/22

o Presentation of the Results

The laser tests comprised measurements both of the entire fluorescence spectrum and of the fluorescent signal around 685 nm. The measuring system was occasionally calibrated against a 10^{-5} M rhodamine solution in a beaker placed immediately above the water surface.

Figure 11 shows a typical fluorescence spectrum from the sea water together with corresponding ones from rhodamine or drinking water in a plastic pail. In general the fluorescence from the sea had its maximum at the interference filter centered at 685 nm (a maximum at 668 nm was established for some of the measurements) and the fluorescence level while using these filters normally surpassed that of the rhodamine. Formation of the means were usually made over 50 pulses/channel at a pulse repeater frequency of 10 Hz.

In order to make a relevant comparison with the manually made measurements of the chlorophyll a concentration, two parameters, S_1 and S_2, were calculated from the data obtained. These were defined according to:

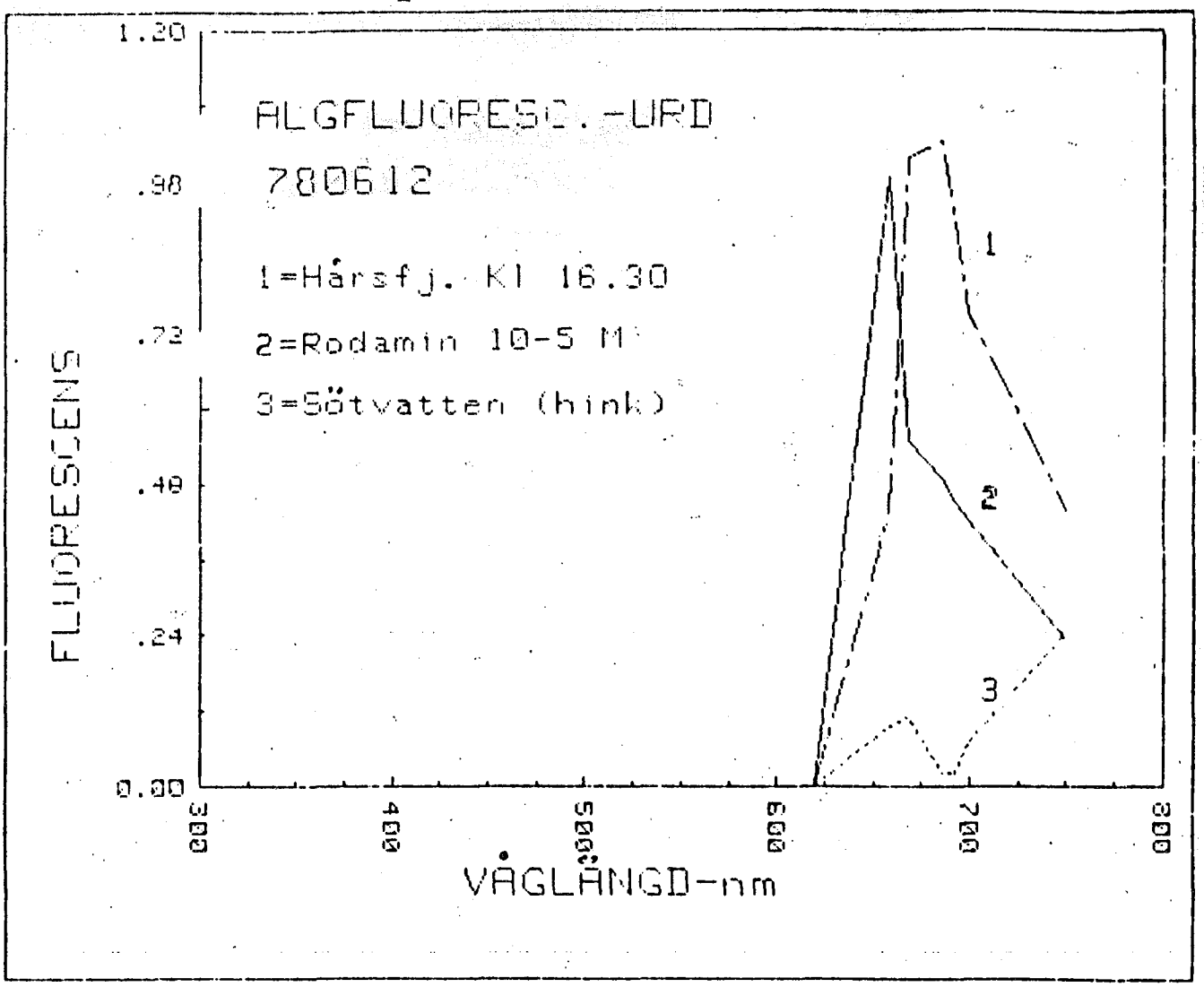


Figure 11. Examples of fluorescence spectra from the sea and from 0.5 l 10^{-5} M rhodamine 6 G as well as from fresh water (free of algae). For each channel, the mean was formed over 50 pulses. Interference transmission was blocked out by a color filter. Laser wavelength: 600 nm.

Key: 1. Algal Fluorescence. - The "Urd", June 12, 1978.
1. Haarsfjaerden, 4.30 pm
2. Rhodamine 10^{-5} M
3. Fresh water (in a pail).
2. Fluorescence
3. Wavelength - nm.

$$S'_1 = \frac{S_m}{S_{rod}} \tag{1}$$

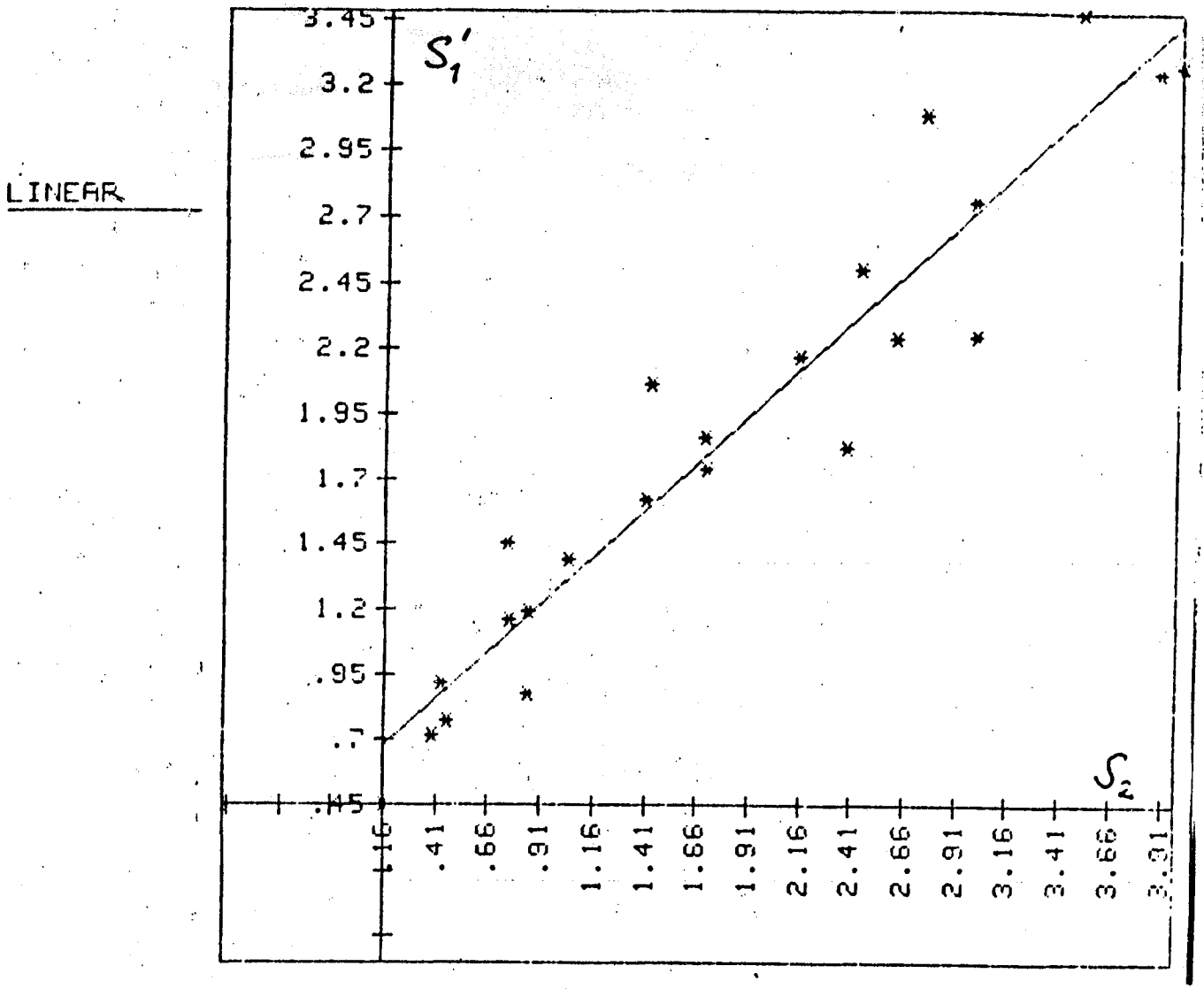
$$S'_2 = \frac{S_m - S_{750}}{S_{750}} \tag{2}$$

where S_m is the maximum fluorescence signal measured (oridnarily at 686 nm), S_{rod} is the rhodamine fluorescence at the same wavelength and S_{750} is the background fluorescence from the sea at 750 nm, considered not to be emanating from the algae. If S'_2 and S_2 are placed in a diagram (Figure 12), it is evident that the dots will with few exceptions group around a straight line. For $S_2 = 0$, this gradient cuts the S'_1 axis at the value = S_{10}, which can be interpreted as background value of S in the absence of any algae. When S'_1 is corrected by subtracting S_{10}, both S'_1 and S_2 ought to be proportional to the induced fluorescence from chlorophyll a. According to [1], the fluorescent effect obtained, P_m, emanating from the algae, can be written:

$$P_m = \frac{K}{R^2} \cdot \sum_j \frac{\sigma_{f,j}(\lambda_\ell) \cdot c_j \cdot P_\ell}{(\alpha_f + \alpha_\ell)} \tag{3}$$

where j is the index of the type of algae; C_j corresponds to the concentration of chlorophyll a; $\sigma(\lambda_\ell)$ is the profile of the fluorescence at λ_ℓ; P is the laser output; K is a systematic constant; R is the distance from the receiver to the water surface; and α_f and α_ℓ, respectively, the exponentially attenuating coefficients of the fluorescence or the laser wavelengths. Although it is possible to make a simplification and enter the mean $\bar{\sigma}_f$ or $\bar{c}$, respectively, for the profile and the chlorophyll concentration, it is obvious from (3) that we have to know $(\alpha_f + \alpha_\ell)$ in order to be able to calculate $\bar{c}$. Since during the test we did not have direct access to the values α_f or α_ℓ, we assumed $(\alpha_f + \alpha_\ell)\alpha \frac{1}{V}$, where V was the opacity depth measured. According to the data above we should expect a relation of the type:

$$\bar{c} \propto \frac{P_m}{\bar{\sigma}_f \cdot P_\ell} \cdot \frac{1}{V} \propto \frac{S_1 \,(el.\, S_2)}{\bar{\sigma} \cdot P_\ell \cdot V} \tag{4}$$



/24

Figure 12. Relationship between the fluorescence signal measured around 686 nm and that of rhodamine (S_1) and a fluorescence signal at 750 nm (S_2), respectively, used as reference data.

i.e., the chlorophyll concentration $\bar{c}$ is proportional to S_1 (or S_2) and at a constant fluorescence signal inversely proportional to the average $\bar{\alpha}_f$, the laser effect P_l and the opacity depth V. /26

For a preliminary comparison between the laser measurements with the manually made tests and the fluorimeter recording, respectively, we used S_1 and S_2 standardized to a measured opacity depth, V. This varied throughout the test period from 2.5 to 7 m. The standard factor was put at 1 for V = 2.5 m.

Results June 13, 1978

The measurements were started at the pier at Berga at 11.00 am and continued via, among others, the Haarsfjaerden and the Toroefjaerden down to Askoe, where the ship anchored at 5.00 pm. Since the laser measurements could not be reliably conducted when cruising due to the foam formation on the surface of the water, a number of stops were made en route for laser tests. The results of these tests are shown in Figure 13. The squares mark the maximum fluorescence in relation to the background fluorescence at 750 nm (S_2) and the filled circles indicate the fluorescence measured in relation to calibration against rhodamine, corrected for background noise according to the data above (S_1). Correction for measured depth of opacity according to the figure has also been made. During all these tests, the laser wavelength was 600 nm just as during the major part of the test period. The reason for this was in part that the laboratory test had shown that 600 nm was an effective wavelength for exciting fluorescence from chlorophyll a, in part that the laser worked more efficiently and at a better effect at this wavelength in comparison with the emission within the UV or the blue.

The solid line in Figure 13 shows the deflection of the SNV fluorimeter as /28
measured on water from a depth of 1.5 m and the crosses (x) and the triangles (Δ) indicate respectively the chlorophyll a concentration of the manual tests at the SNV and the Askoe laboratories. The concentration of chlorophyll throughout the day was between 1 and 2 mg/m^3. There is a positive correlation between the values of the laser and the others. In addition, the numerical values of S_1 and S_2 correspond directly to the chlorophyll concentration as measured and expressed in mg/m^3. The correlation coefficient between the latter and the laser data (S_1) was calculated to 0.57 (7 test points), between the fluorimeter data and S_1 it was 0.80 (from 20 points) and between chlorophyll determinations and the fluorimeter data 0.43 (on 10 points).

Figures 14 and 15 illustrate some spectral recordings. At 750 nm, the background fluorescence varied fairly little throughout the test period and was at a low level compared with the fluorescence from the rhodamine at 685 nm.

An analysis of the species composition and the biomass was made at the SNV on two occasions and can be seen from Appendix II. As is obvious, the portion of bluegreen and green algae is low while the bulk consists of Pyrrophyta (armored

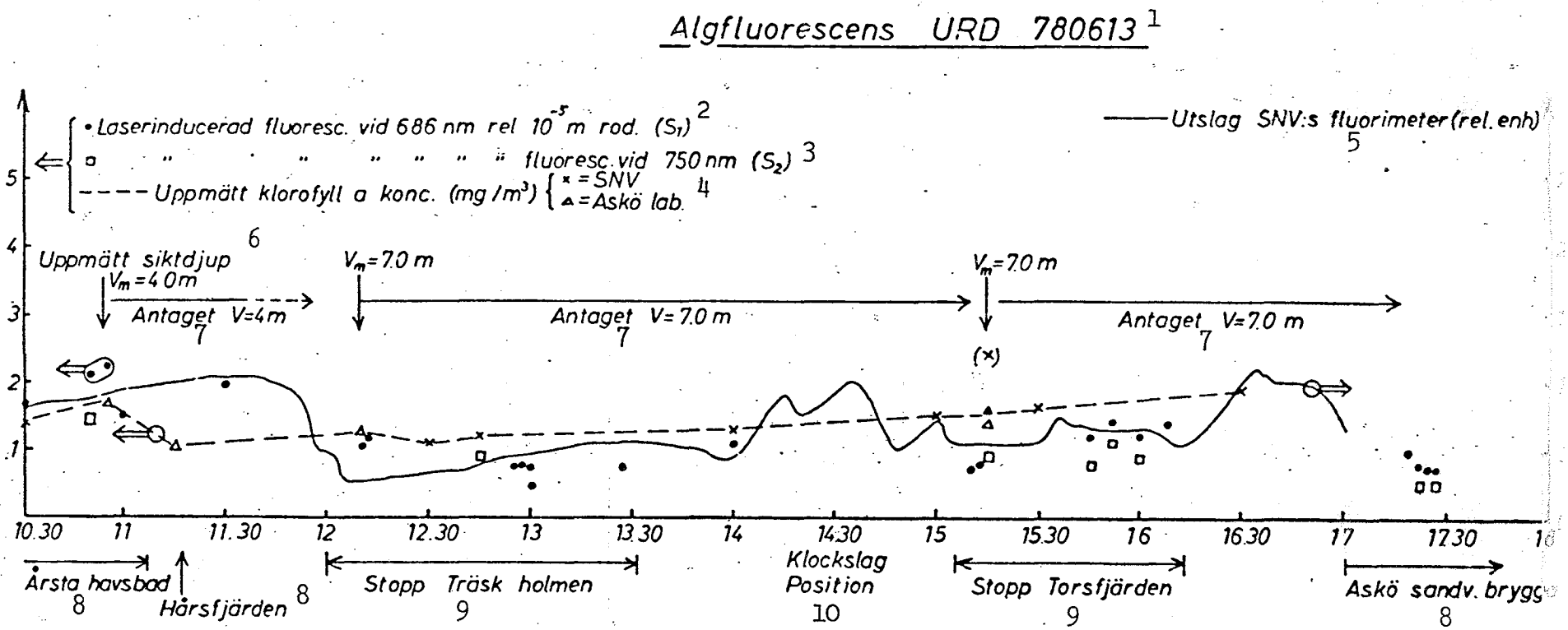
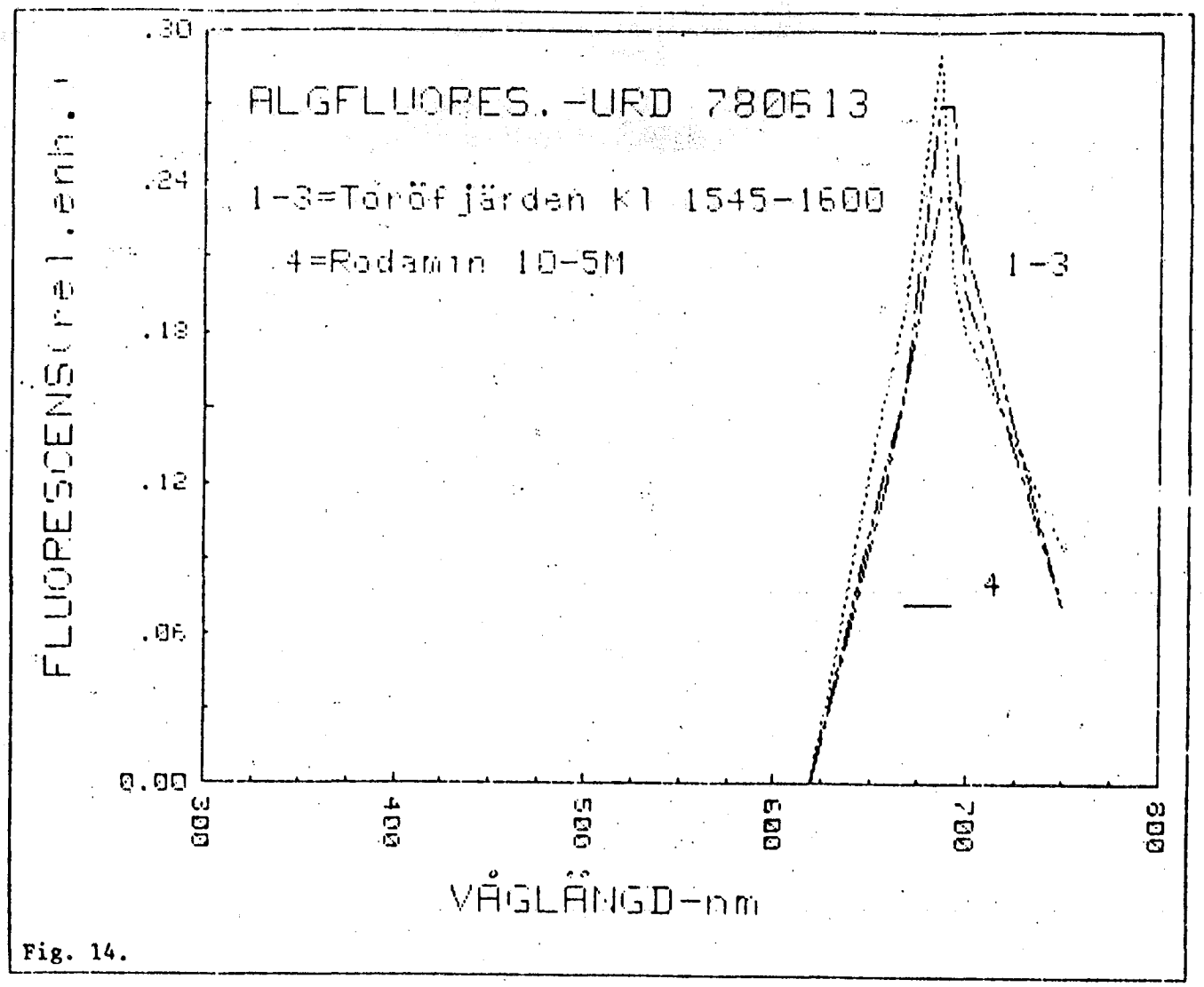


Figure 13. Compilation of data from laser and other measurements made. Note that the laser data are designated by S_1 and S_2, which cannot be be directly converted into chlorophyll concentration.

Key: 1. Algal fluorescence, the "Urd", June 13, 1978.
2. ● Laser induced fluorescence at 686 nm in rel. to 10^{-5} rhodamine (S_1)
3. □ Laser induced fluorescence at 686 nm in rel. to fluorescence at 750 nm (S_2)
4. - - - Chlorophyll a concentration measured at respectively the SNV (x) and the Askoe (Δ) laboratories.
5. ——— Deflection of the SNV fluorimeter (rel. units)
6. Opacity depth measured
7. Opacity depth assumed to be V = 4m (7.0 m)
8. Place names (see map)
9. Stop at Traeskholmen (Torsfjaerden).
10. Time, position.



/29

Figure 14. Examples of spectral recordings made on June 13.

Key: 1. Algal fluorescence. - The "Urd", June 13, 1978

1-3. Toroefjaerden 3.45 - 4.00 pm

2. Rhodamine 10^{-5} M

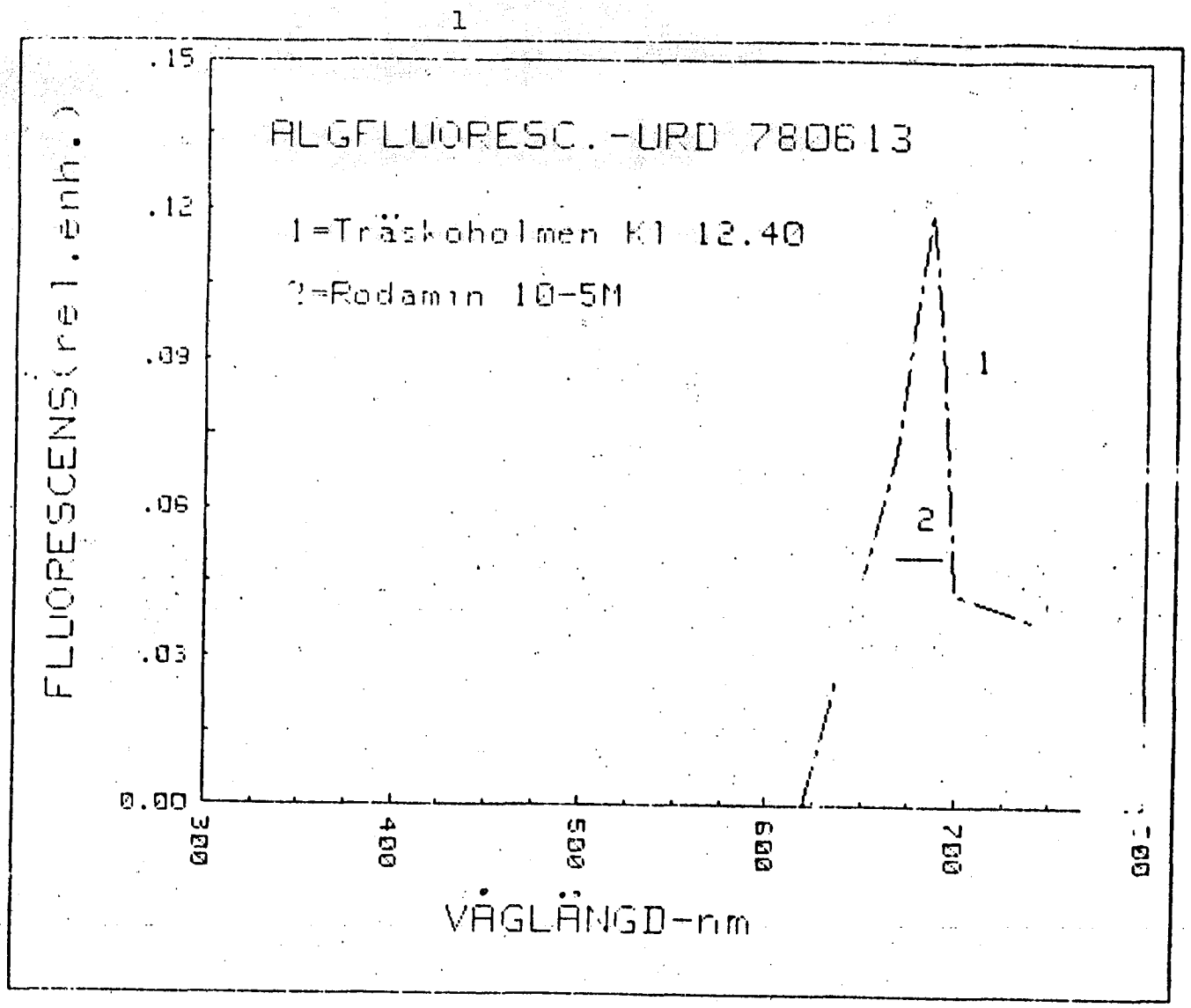
2. Fluorescence (rel. units)

3. Wavelength - nm.

flagellates) and diatoms. Unfortunately, it had to be assumed that the sensitivity to excitation at 600 nm is fairly low for these taxa (cf. Figures 3, 4 and 8).

Results June 14, 1978

The measurements started at Askoe during the morning and continued through Regarn, Oaxen, Skanssundet, AEngsholmen and eastern Himmerfjaerden until we anchored /31



/30

Figure 15. Examples of spectral recordings on June 13.

Key: 1. Algal fluorescence - The "Urd" , June 13, 1978

1. Traeskoholmen 12.40 pm
2. Fluorescence (rel. units)
3. Wavelength - nm.

in the Kaggfjaerden. During this day, the maximum chlorophyll concentration measured was 6 mg/m^3.

It is evident from Figure 16 that the laser measurements agree fairly well with the chlorophyll measurements. The broad scattering of the laser data, especially around 1.00 pm, was explained by the fact that we had some difficulties with the

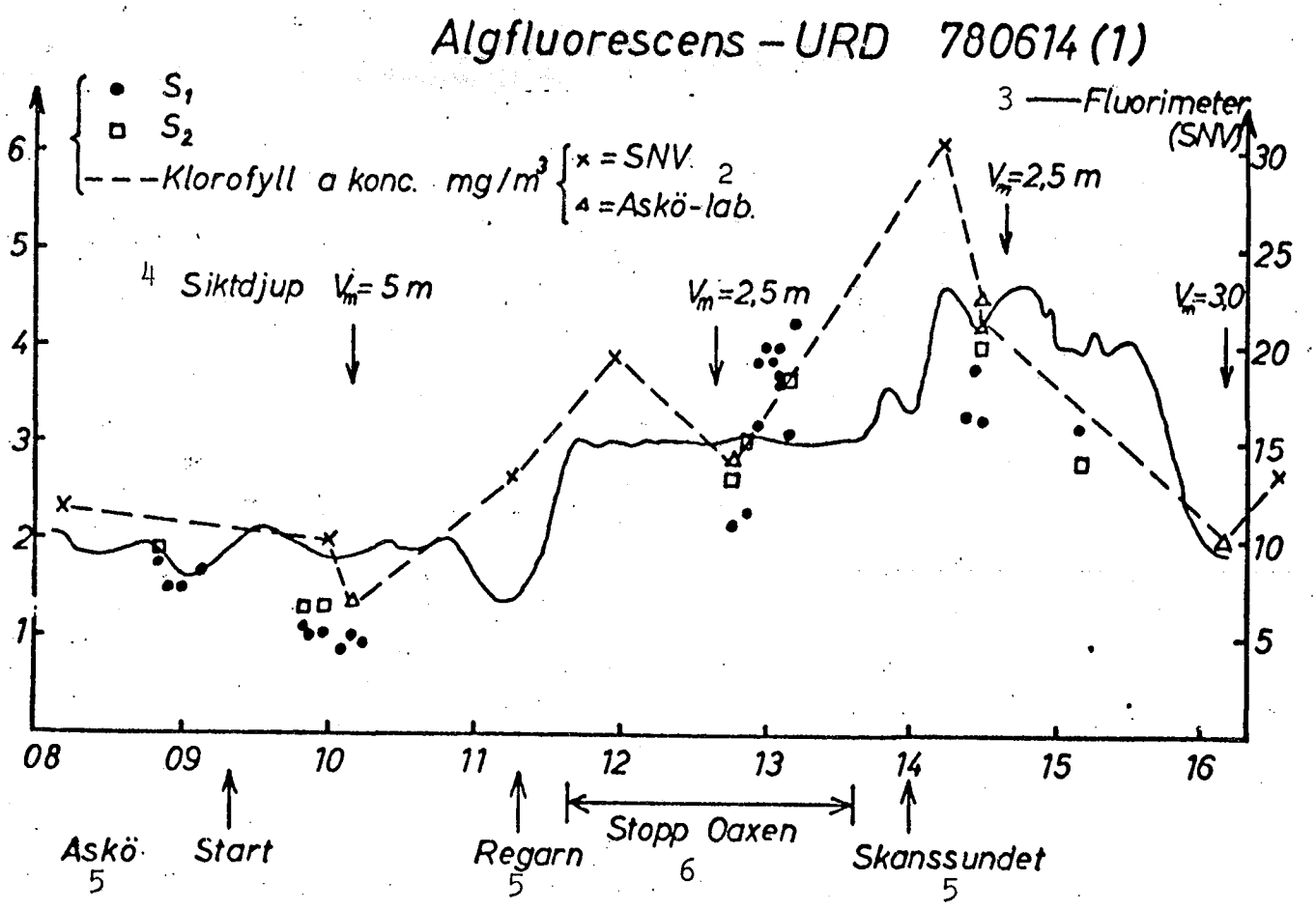
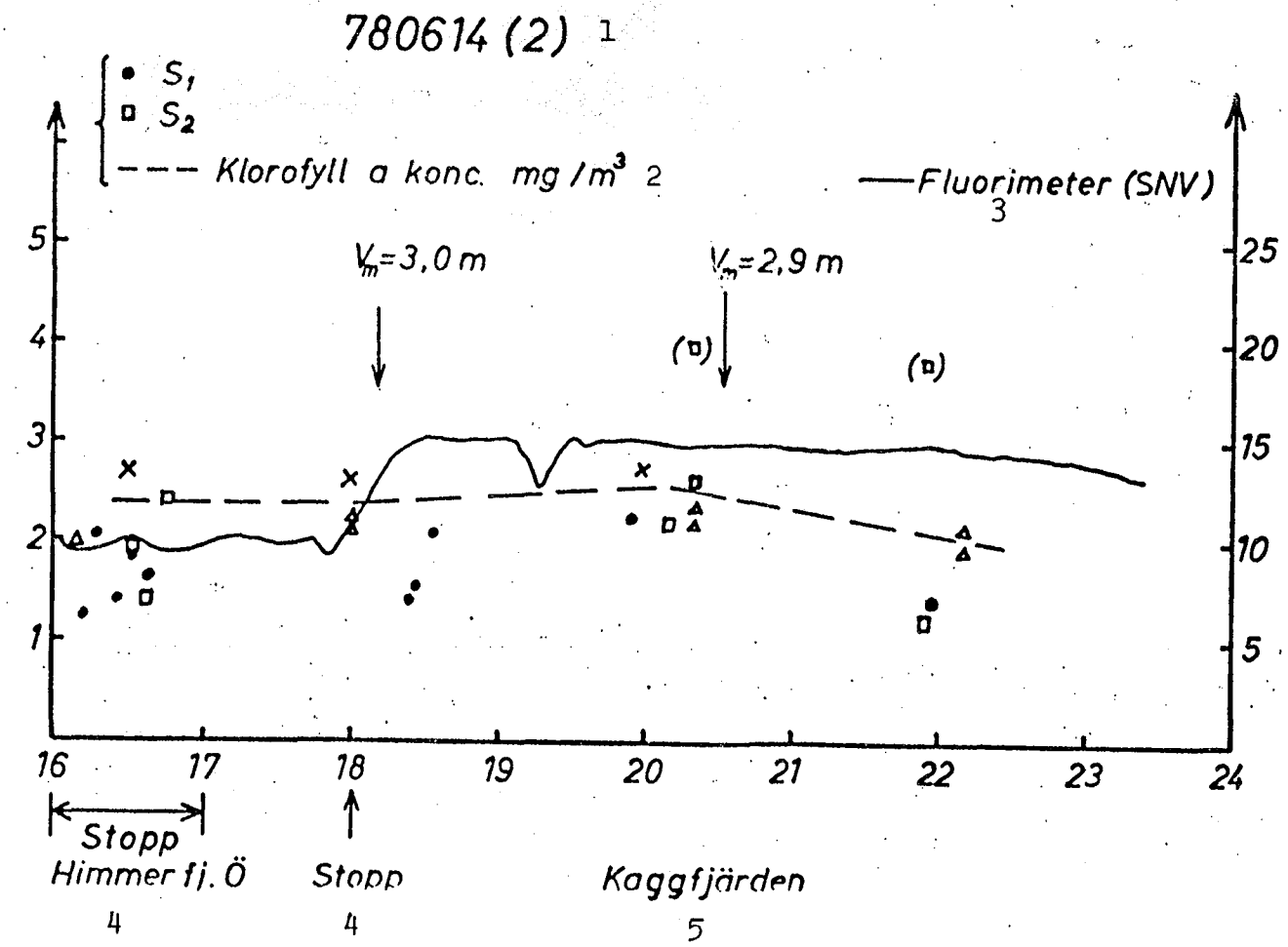


Figure 16 a. Compilation of measurements made on June 14. The broad scattering of the laser data around 1 pm is assumed to emanate from disturbances caused by reflected sunlight.

Key: 1. Algal fluorescence - The "Urd", June 14, 1978
2. Chlorophyll a concentration, mg/m³, x SNV, Δ Askoe laboratory.
3. SNV fluorimeter
4. Opacity depth V_m = 5 m (2.5 m)
5. Place names
6. Stop at Oaxen.

sunlight being directly reflected into the receiver at certain positions, causing /31
trouble with the photomultiplier. Later, the fluorescence signal was calibrated against the opacity depth as measured. Except for the first case at a depth of 5 m, this was found to be at between 2.5 and 3.0 m.

Examples of the spectral analyses are given in Fig. 17. Here the ultimate filter was changed from being a blocking filter to a 795 nm filter. The background fluorescence at 795 nm is, as can be seen, very low. It is evident that the main part of the algae also here belong to taxa of Diatomae and Pyrrophyta.



/33

Figure 16 b. Compilation of measurements made on June 14. The broad scattering of the laser data around 1 pm is assumed to emanate from disturbances caused by reflected sunlight.

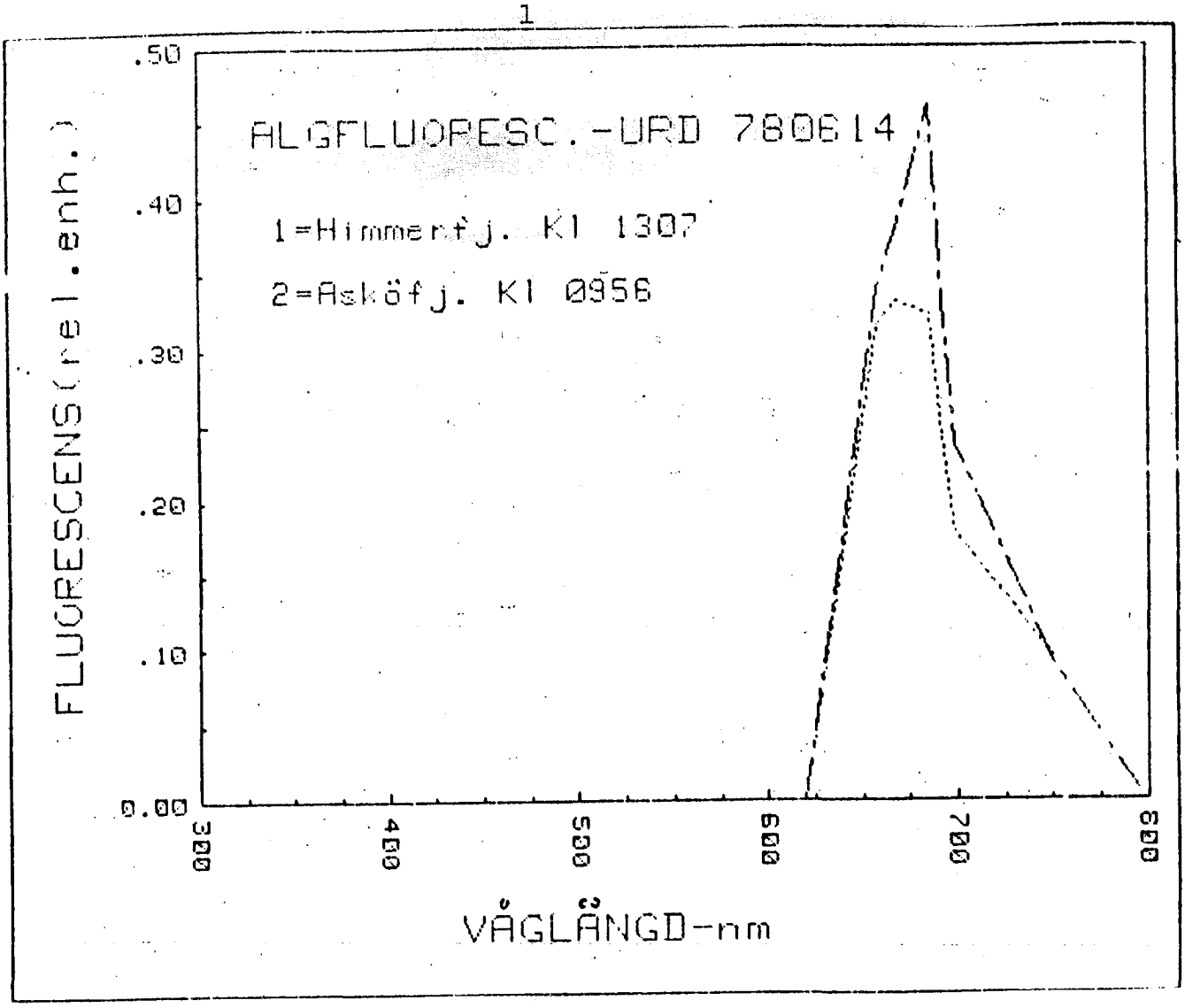
Key: 1. June 14, 1978 (2)
2. Chlorophyll a concentration, mg/m^3
3. SNV fluorimeter
4. Stop at Himmerfjaerden
5. Place name.

Results June 15, 1978

/31

We anchored in the Kaggfjaerden from 6.00 pm the previous day until 8.15 on June 15. Measurements made during the night comprised excitation at 300 nm and 490 nm. In the morning, we switched the course of the beam, previously going via the mirror over the railing to the water, to make measurements in a recess in order to enable us to make continuous measurements when cruising.

1



/34

2

3

Figure 17. Fluorescence spectra taken on June 14.

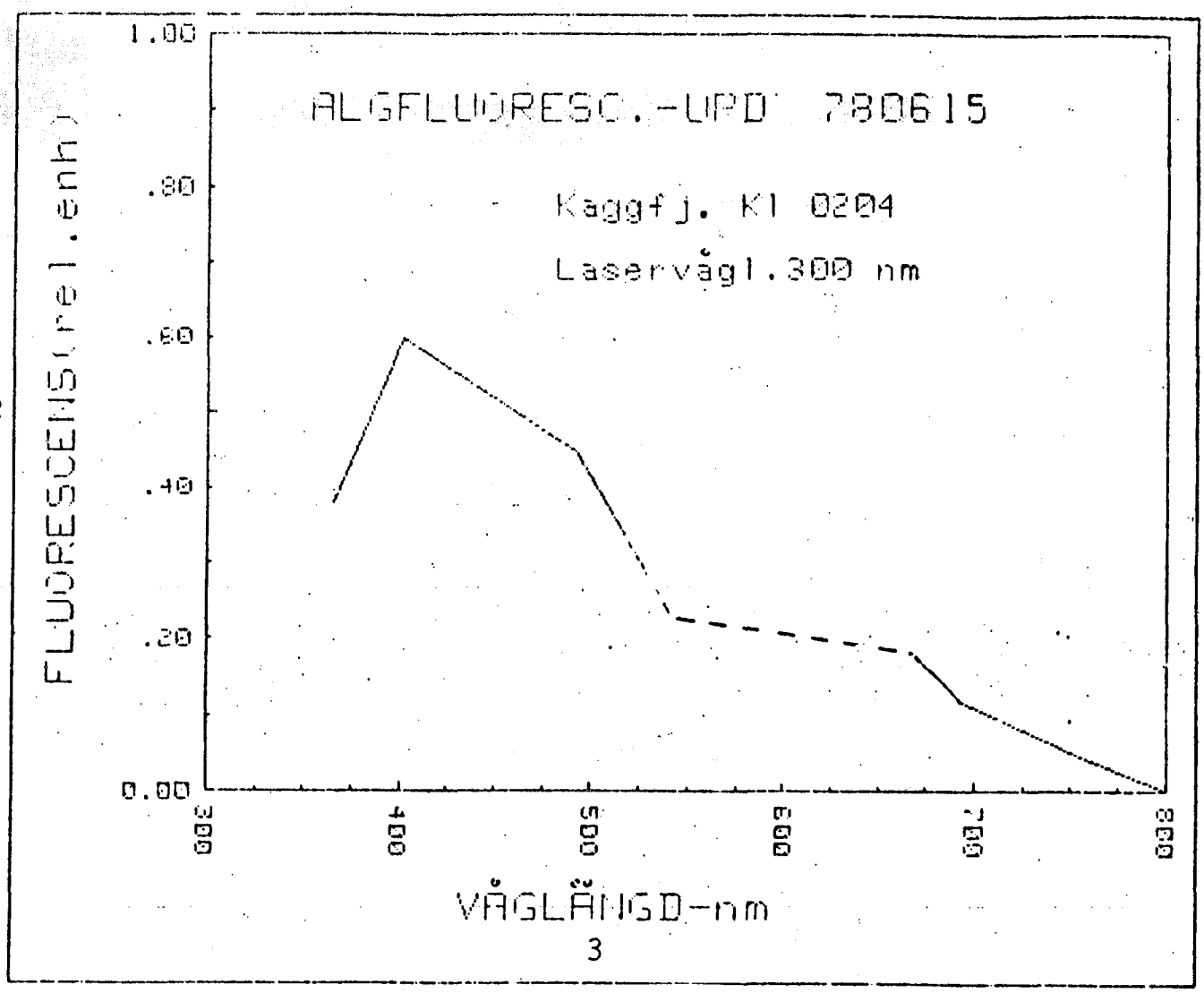
Key: 1. Algal fluorescence, - "Urd", June 14, 1978
1. Himmerfjaerden 1.07 pm.
2. Askoefjaerden 9.56 am.

UV Measurements

/35

By doubling the frequency of the yellow laser light at about 600 nm, it could be converted to 300 nm at a cost of the reduction by a factor of 10 of the output effect. The fluorescence spectrum of the sea at 300 nm excitation is illustrated in Figure 18.

The response of the blue range of the wavelength, not emanating from the algae, in this case definitely exceeds the signal at 685 nm. This amounted to only 1/40th of the corresponding figure for excitation at 600 nm which, thus, is 4



/36

Figure 18. Fluorescence spectrum from the Kaggfjaerden. Excitation length: 300 nm.
Key: 1. Algal fluorescence - The "Urd", June 15, 1978
1. Kaggfjaerden 2.04 am
2. Laser wavelength 300 nm.
2. Fluorescence (rel. units)
3. Wavelength - nm.

times more effective if the difference in laser effect is taken into consideration. /35
On the other hand, the UV light evokes a strong fluorescence from, e.g., oil, which was demonstrated by a few simple experiments (Figure 19).

Excitation at 490 nm.

Laser excitation at 490 nm proved more effective than at 600 nm. Unfortunately,

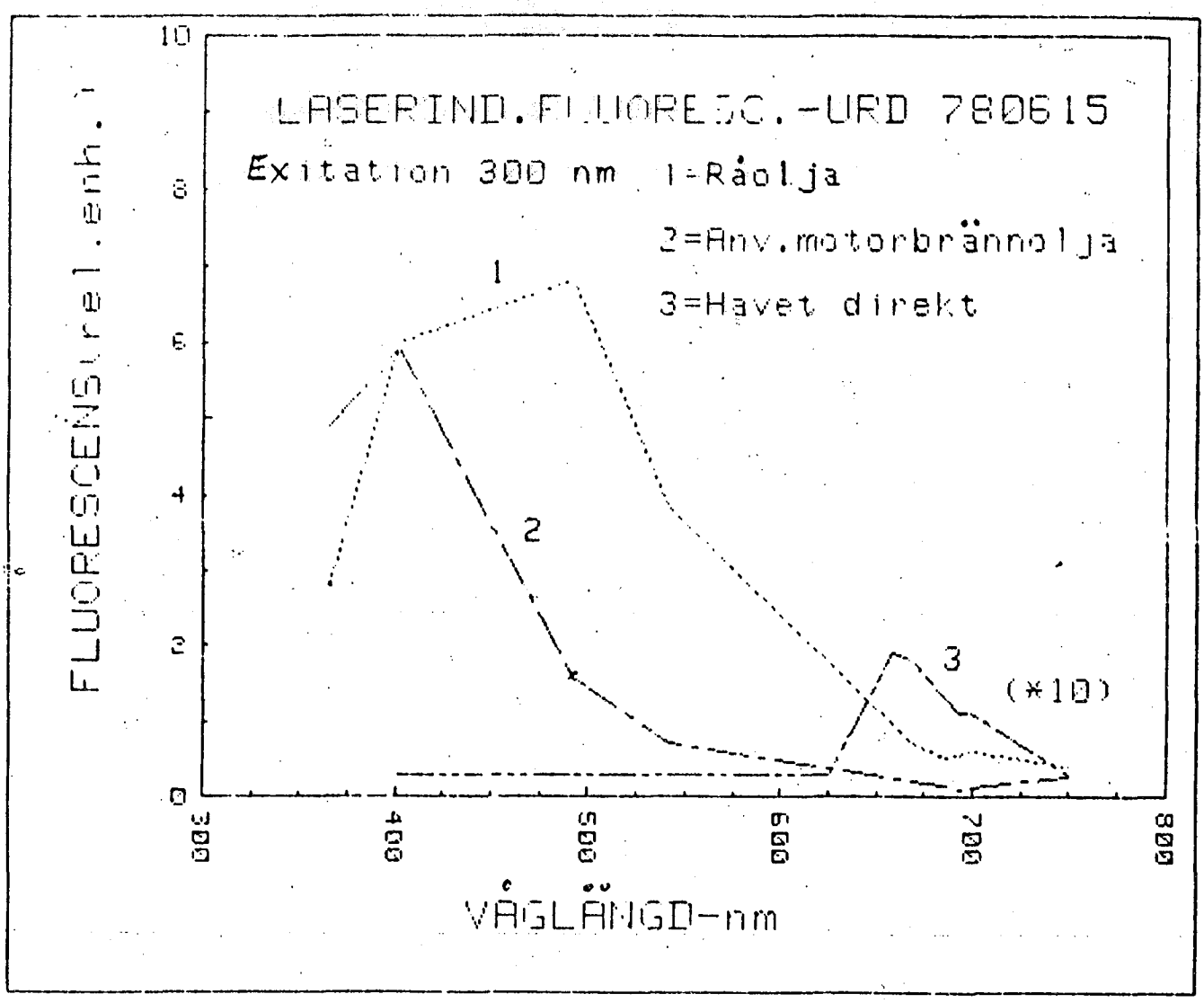


Figure 19. Fluorescence spectra from oil and the sea. In the latter case, wavelength below 600 nm were blocked by color filters. Note the great difference in fluorescent emission between water and oil.

Key: 1. Laser induced fluorescence - The "Urd", June 15, 1978
Excitation 300 nm. 1. Crude oil
2. Used engine oil
3. Sea water alone
2. Fluorescence (rel. units)
3. Wavelength - nm.

the stability and duration of the laser emission is unsatisfactory for flashbulb pulsed dye lasers within the blue range. This explains why after a test period of a few hours we returned to the 600 nm excitation. /35

Figure 20 provides a comparison between fluorescence at 490 and 600 nm.

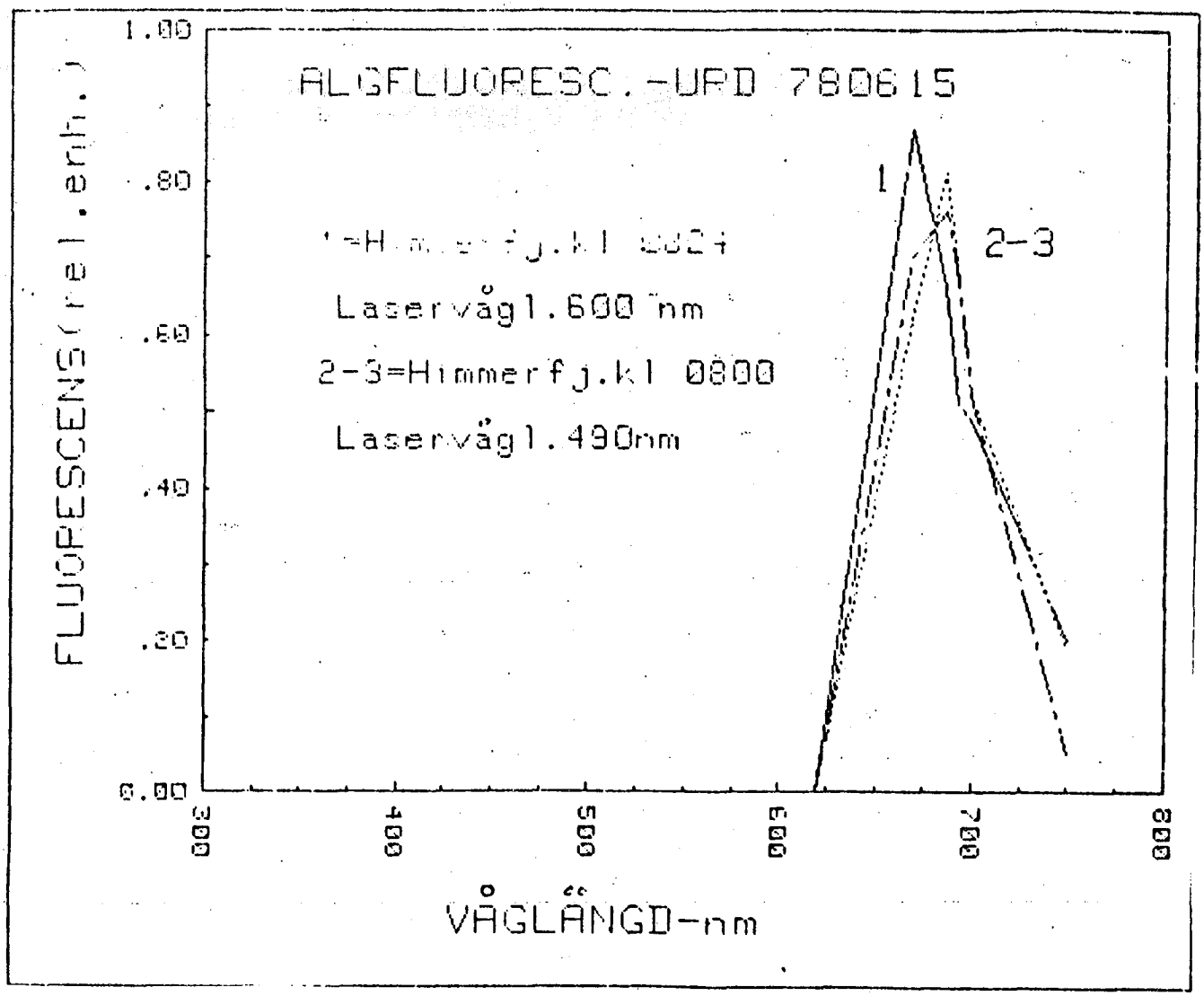
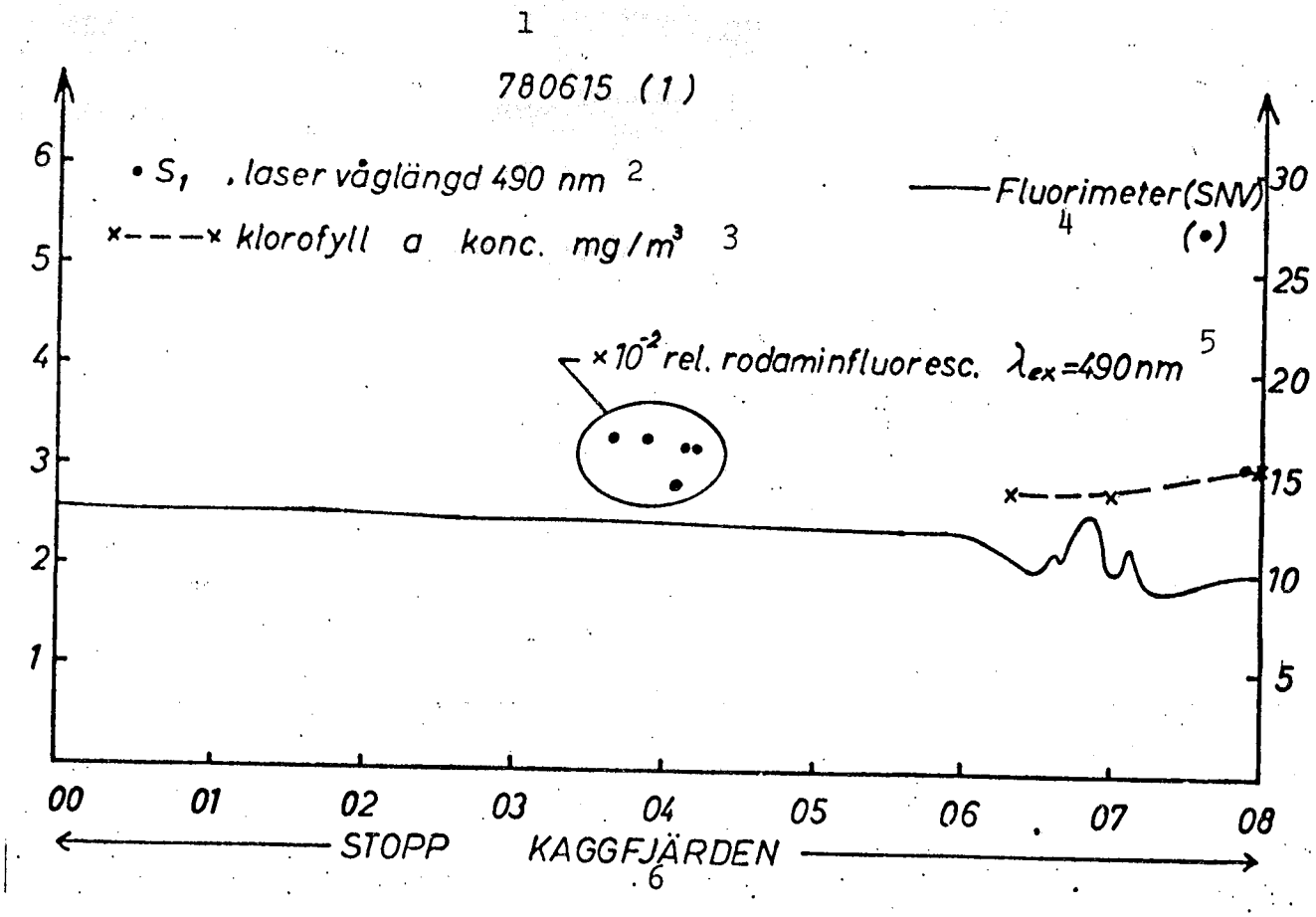


Figure 20. Comparison between excitation at 600 nm and 490 nm, respectively.

Key: 1. Algal fluorescence - The "Urd", June 15, 1978
1. Himmerfjaerden 11.24 pm
Laser wavelength 600 nm
2-3. Himmerfjaerden 8.00 am
Laser wavelength 490 nm.
2. Fluorescence (rel. units)
3. Wavelength - nm

The laser effect at 600 nm was about twice that at 490 nm, a fact which we had not taken into consideration during the recording because the reference-sensing laser detector had to be adjusted when switching wavelengths. The signal level was then set at the same level as for 600 nm in order to maintain the optical dynamics. We want to draw attention to the facts that the fluorescence maximum



/39

Figure 21 a. Compilation of measurements made on June 15. Some measurements are shown relating to corresponding rhodamine fluorescence at the excitation wavelength 490 nm.

Key: 1. June 15, 1978 (1)
2. S_1, laser wavelength 490 nm
3. x- - -x chlorophyll a concentration, mg/m^3
4. SNV_2 fluorimeter
5. 10^{-2} rhodamine fluorescence, λ_{ex} = 490 nm
6. Stop in the Kaggfjaerden.

was displaced from 668 nm to the 686 channel and that the background level at 750 nm is considerably lower during excitation at 490 nm. /35

Measurements made in a Recess /42

In order to allow ourselves to make continuous measurements and to avoid reflected sunlight, some experiments were made in a recess (see Figure 9). The laser wavelength was 600 nm.

The results are given in Figures 21 b. Note that the laser data agree well with the other data, especially during the significant change in chlorophyll

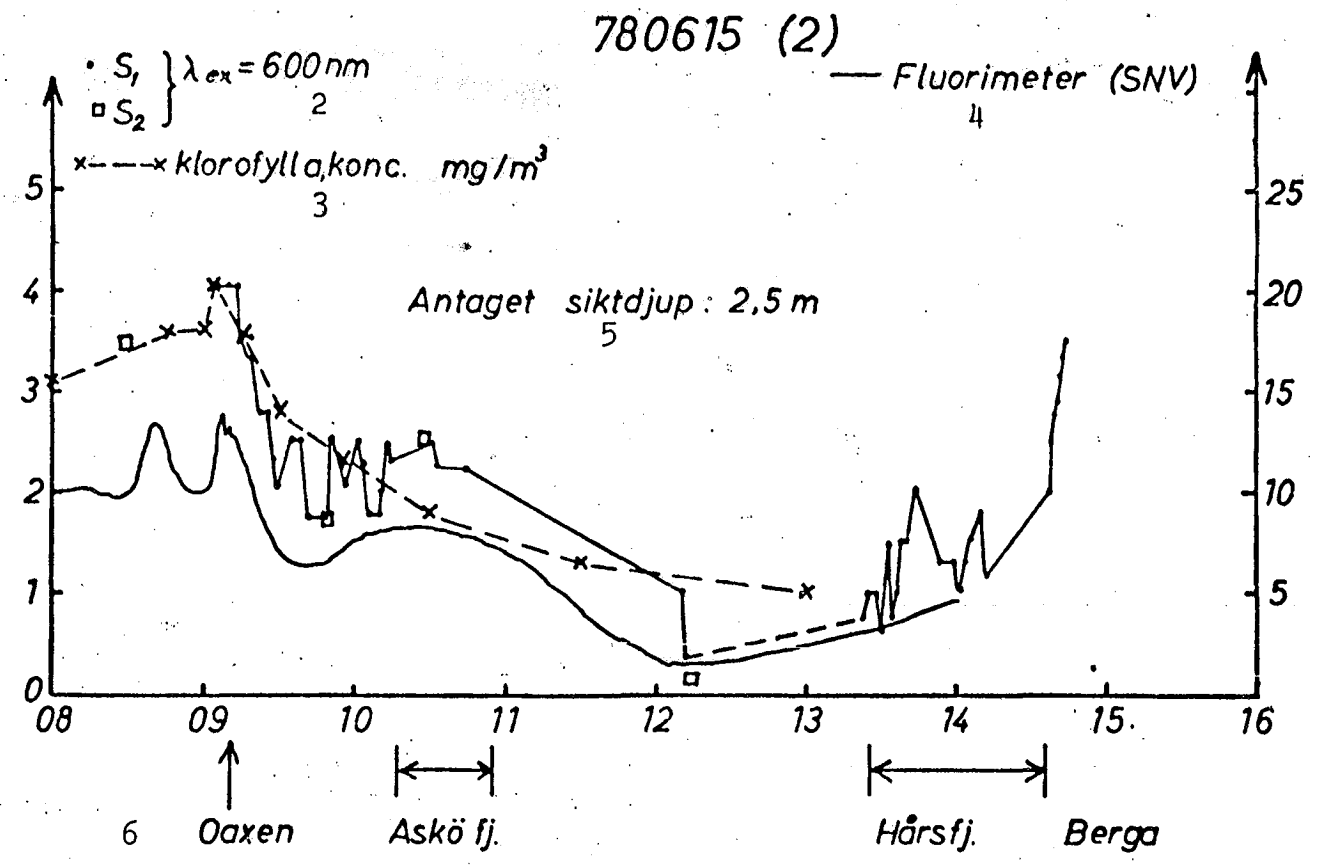
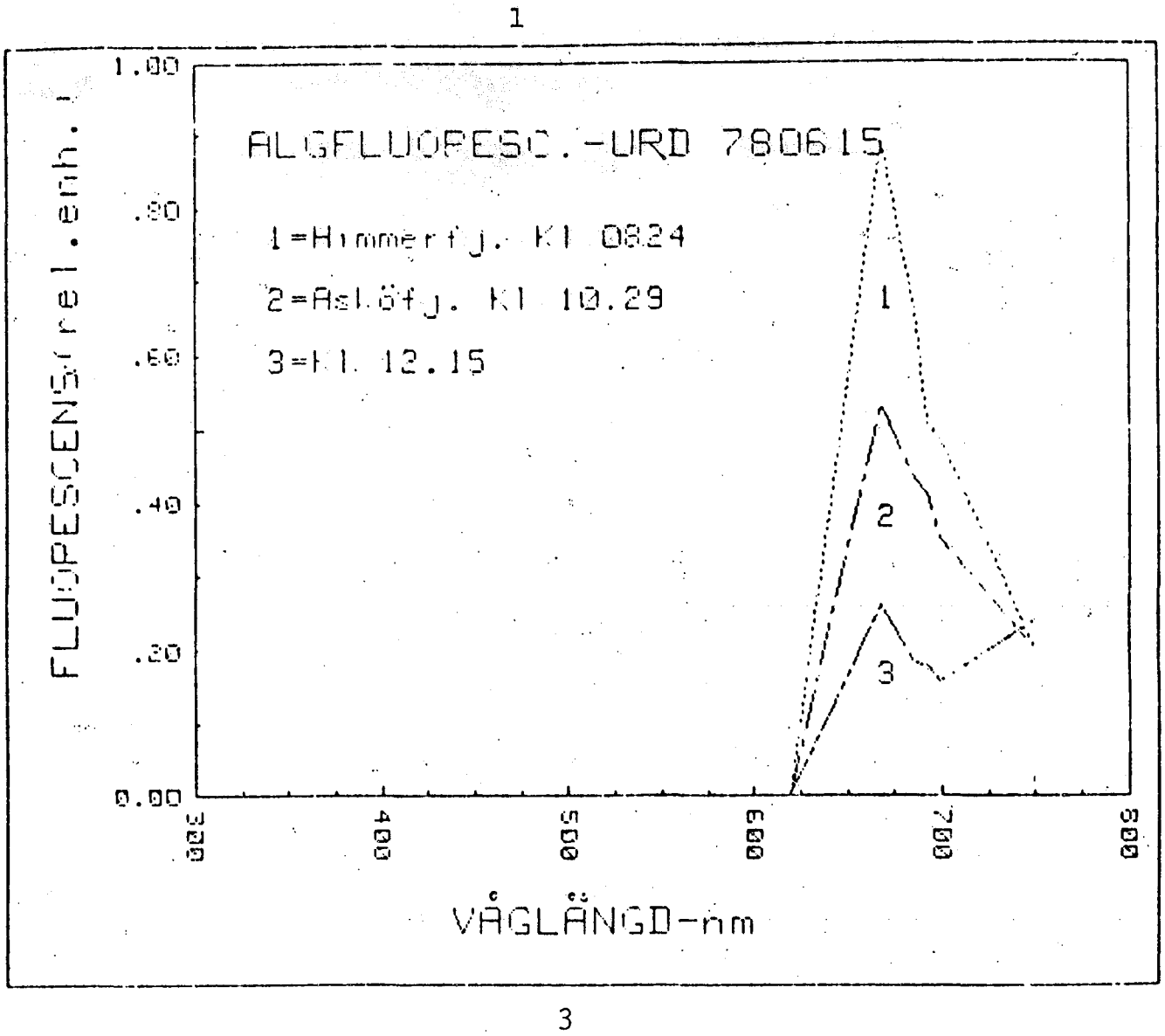


Figure 21 b. Compilation of measurements made on June 15. The results from measurements in a recess are illustrated.

Key: 1. June 15, 1978 (2)
2. S_1 and S_2, λ_{ex} = 600 nm
3. x - - - x chlorophyll a concentration, mg/m^3
4. SNV fluorimeter
5. Assumed opacity depth
6. Place names.

concentration between 9 o'clock and noon. Unfortunately, there are no data on the opacity depth during these measurements, but an opacity depth of 2.5 m was assumed. Figure 22 furnishes some fluorescence spectra, reflecting the variation mentioned. Spectrum no. 3 (12.15 pm) gives the lowest fluorescence response measured with a peak value (at 668 nm) just exceeding the background value at 750 nm.



/40

Figure 22. Examples of fluorescence spectra made on June 15.

Key: 1. Algal fluorescence - The "Urd", June 15, 1978
1. Himmerfjaerden, 8.24 am
2. Askoefjaerden 10.29 am
3. At 12.15 pm.
2. Fluorescence (rel. units)
3. Wavelength - nm.

Numerical Evaluation of a Profile of Fluorescent Data on the Basis of Test Data /43

In this chapter we will try to find a numerical value of the profile of fluorescence data for chlorophyll a, based on the measured cases when an analysis of the algal composition has been made.

Since we used a laser wavelength (at 600 nm), we are unable to distinguish

the contributions from different color groups and the profile must be taken as a mean of these. In order to obtain an idea of the uncertainty of the procedure, an evaluation can be made in two ways:

A. by using the fluorescence signal measured from rhodamine, the profile of which had been measured in the laboratory [2], or

B. by direct recalculation to emitted fluorescent effect of the signal obtained.

A. According to (3) the mean profile of fluorescence from chlorophyll a can be written:

$$\bar{\sigma}_{alg} = \frac{P_{fl.alg}}{P_{fl.rod}} \cdot \frac{V_{rod}}{V_{alg}} \cdot \frac{1}{C_{alg}} \cdot \bar{\sigma}_{rod\ (\Delta\lambda_f)} \tag{5}$$

where P is the fluorescent effect at 686 nm; C_{alg} is the concentration of chlorophyll a in mg/m^3; V is the effective volume from which the fluorescence is excited; and $\bar{\sigma}_{rod}$ is the rhodamine fluorescence at a particular optic band width, e.g., 1 nm. If the laser beam can be considered parallel in this connection, it is valid that $\frac{V_{rod}}{V_{alg}} = \frac{Z_{rod}}{Z_{alg}}$ where Z is the height of the column of liquid. We used the measured opacity depth for Z_{alg}, but as a value for Z_{rod} we used 5×10^{-2} m.

According to (2), it is valid that $\bar{\sigma}_{rod} = 1.64 \times 10^{-22}\ m^2$. If these data /44 are used, $\bar{\sigma}_{alg}$ can be calibrated against $\Delta\lambda = 1$ nm according to column 1 in Table II.

B. In this case we used the equation for the obtained fluorescence effect [2], whereby:

$$\bar{\sigma}_{alg} = P_{fl} \cdot \frac{2R^2 (\alpha_f + \alpha_l) \cdot \Delta\lambda_f}{\varsigma \cdot A \cdot \tau_f \cdot C_{alg} \cdot P_L \cdot \tau_{VL}\ \Delta\lambda_D} \cdot \left(\frac{\theta_l}{\theta_m}\right)^2 \tag{6}$$

where R is the distance between the receiving optic and the water surface; $\alpha_{f,\ l}$ is the attenuating coefficient at fluorescent or laser wavelengths; $\Delta\lambda_{f,\ D}$ is the half-width of the filter or of the fluorescent emission; ς corresponds to the optic efficiency; A is the surface of the receiving aperture; T_f is the transmission of the interference filter; P_L the laser effect emitted; T_{Vl} the loss of transmission at the water surface and $\theta_{l,m}$ is the divergent angle

of the laser beam or of the receiver. P_{fl} is obtained from

$$P_{fl} = \frac{U}{R \cdot S} \qquad (7)$$

where U is the pulse peaks (Volts) on the oscilloscope above the load resistance R (R = 1 kΩ) and S is the sensitivity of the photomultiplier in A/W. The latter was measured in the laboratory to 50 A/W at a cathode voltage of 2 kV and to 9.2 A/W at 1.5 kV. If the remaining parameters are entered into (6), we obtain σ_{alg} according to column 2 in Table II. These data contain most likely more errors than those calculated according to (A), because some of the parameters, (e.g., P_L) could not be explicitly measured in every case.

TABLE II

/45

EVALUATION OF PROFILES OF ALGAL FLUORESCENCE AT 686 nm.

Time and place *	σ_{alg} calculated per molecule and (calibrated against $\Delta\lambda$ = 1 nm)**	
	According to (A)	According to (B)
June 13, 1978, 1.27 pm (Traeskoholmen-Mellsten)	11×10^{-24} m^2	5×10^{-24} m^2
June 13, 1978, 3.46 pm (Toroefjaerden)	14×10^{-24} m^2	8×10^{-24} m^2
June 14, 1978, 12.48 pm (Oaxen)	21×10^{-24} m^2	12×10^{-24} m^2

* Analysis of algal composition, see Appendix II.

** 1 mg chlorophyll a corresponds to ca. 6.8×10^{17} molecules.

According to the analyses of the algal composition during these measurements/45 (cf. Appendix II), the armored flagellates and the diatoms dominate. In [2] the profiles of two taxa from these algal groups were measured to

$\sigma = 3 \times 10^{-24}$ m^2 (Peridinium cinctum, armored flagellate),

$\sigma = 1.5 \times 10^{-23}$ m^2 (Asterionella, a diatom)

5. CONCLUSIONS

/46

The experiments performed show a relatively satisfactory agreement between laser induced fluorescence measured at about 685 nm and the result obtained for chlorophyll a concentration during manual testing. During the evaluation, a comparison was made between the fluorescent signal measured about 685 nm and calibrated against a corresponding fluorescent signal from a reference solution of 10^{-5} M rhodamine 6 G or calibrated against fluorescence measured at 750 nm. A satisfactory correlation was obtained between both of these as well as the manually measured concentration of chlorophyll a.

No attempts were made to determine the absolute chlorophyll concentration on the basis of the laser data, since information on the algal composition was lacking for the major part of the experiment.

It was furthermore doubtful whether the fluorescence profile of freshwater algae, obtained in the laboratory, could be used here. The analyses made by the Nature Conservancy Board (SNV) showed for some cases measured that the algae were composed mainly of armored flagellates and diatoms. According to laboratory tests, the fluorescence profiles at 685 nm of these compounds could be estimated to within a range from 1 to 20 x 10^{-24} m^2/molecule, nm.

In general, the excitatory laser wavelength was 600 nm. During shorter periods, excitation at 300 nm and 490 nm was also tested. Of these two, 300 nm was less effective than 600 nm in respect to the differences in laser effect, while 490 nm gave rise to about twice as strong a fluorescence as at 600 nm.

In conclusion, the results obtained clearly show that more tests should be /47
made. The following points should then be noted:

- Absolute determinations of chlorophyll a concentration by means of remote sensing laser analysis is difficult, because knowledge of the algal composition and its profile are necessary. For air-borne tests, it is recommended that a number of check points for manual testing are included within the area surveyed.
- In order to increase the accuracy of the measurements, it may be a good idea to calibrate the signal from the chlorophyll fluorescence against the raman signal from the water. The latter is directly proportional to the water volume penetrated.

- In order to obtain a proper raman signal for the calibration, an excitatory wavelength shorter than 600 nm should be selected, e.g., 440 nm, which is coincident with the absorption of chlorophyll a. That wavelength should also be more effective than 600 nm for excitation of the diatoms and the armored flagellates.
- Optic multichannel methods should be used in order to reduce the measuring time.
- By means of simple field tests, an empirical basis should be created for a profile of the common composition of the algae in the Baltic Sea.
- It is desirable to acquire practical experience of air-borne tests in order to be able to learn quickly what effects this procedure may have on the equipment used.

REFERENCES

/48

1. Mumola, P.B., O. Jarret Jr. and C.A.Brown Jr., "Multiwavelength Laser Induced Fluorescence of Algae in-vivo: A New Remote Sensing Technique", Proceedings of the 2nd Joint Conference on Sensing of Env. Pollutants, Washington, D.C., Dec. 10-12, 1973.

2. Sollander, S. and K. Carlson, "Laserinducerad fluorescence fraan alger," [Laser induced fluorescence from algae], FOA-report D-30095-E1, Aug. 1978.

3. Celander, L., K. Fredriksson, B. Galle and S. Svanberg, "Investigations of laser-induced fluorescence with applications to remote sensing of environmental parameters," Chalmers GIPR-149, February 1978.

APPENDIX I

/49

Data on the optical filters used:

Filter Nr	λ_{MAX} (nm)	T_{MAX}	Half-width B (nm)	$B \cdot T_{MAX}$	Spectral sensitivity photomult. PMT FACTOR	T RG 5 Nr 1	T RG 2 Nr 2	F_K'' RG 5(2)	F_K'' RG 2(5)	T RG 2 Nr 2
1	402.5	0.362	10.6	3.37	0.981	0	0	-	-	0
2	492.0	0.33	9	2.97	0.981	0	0	-	-	0
3	541.8	0.34	12.6	4.28	0.943	0	0	-	-	0
4	658.5	0.32	9.4	3.01	0.830	0.13	0.84	0.325	2.10	0.84
5	668.0	0.38	9.6	3.65	0.811	0.57	0.88	1.69	2.60	0.88
6	686	0.72	17.5	12.6	0.792	0.87	0.895	8.68	8.93	0.895
7	692	0.67	18.5	12.4	0.774	0.88	0.897	8.45	8.61	0.896
8	699	0.39	9.5	3.71	0.755	0.89	0.897	2.49	2.51	0.898
9	750.8	0.376	11.5	4.32	0.698	0.89	0.90	2.68	2.71	0.900
10	800 (SVART)	-	-	-	(0.623)	0	0	-	-	0
10A	795	0.38	9	3.42	0.64		0.90		1.97	0.90
10B	777	0.40	10.5	4.20	0.64		0.90		2.42	0.90

Remarks. RG 2 and 5 are color filters. F_K'' is the calibration factor by which the signal is corrected when plotting the fluorescence spectra.

APPENDIX II

/50

National Swedish Nature Conservancy Board
The Research Laboratory
Bruno Bjoernborg.

Algal composition and biomass of samples from the chlorophyll projects, June 13 - 15, 1978

Abbreviations of phyla, etc.:

B = Cyanophyta, bluegreen algae
C = Chrysophyta, "Golden algae"
G = Chlorophyta, green algae
P = Pyrrophyta - armored flagellate (among others)
K = diatoms

Determinations made by Gunnar Guzikowsky.

Species	Type	Biomass mg/m^3	%
MELLSTEN (MYSINGEN), June 13, 1978, 1.45 pm.			
Conyaulax calenata	P	89	42
Gymnodinium - Peridinium - 25 µ	P	32	15
Monads	C	30	14
Ampidinium spp. - 20 - 30 µ	P	15	7
Dinobryon spp.	C	11	5
Rhodomonas pusilla	P	9	4
Skeletonema (cells)	K	8	4
Gymnodinium - Peridinium - 12 µ	P	6	3
Diatoma elongata	K	4	2
Pyramimonas minusculum	G	2	1
		211	99

Species	Type	Biomass mg.m^3	%
TOROEFJAERDEN, June 13, 1978, 3.30 pm.			
Gymnodinium minutum	P	38	21
Pyramimonas	G	36	20
Gymnodinium - Peridinium - 15 µ	P	25	14
Dinobryon balticum-type	C	18	10
Cryptomonas (small)	P	16	9
Dinophycis	P	13	7
Melosira islandica ?	K	13	7
centric Diatom	K	11	6
Pyramimonas minusculum	G	5	3
Rhodomonas pusilla	P	3	2
		178	99
OAXEN, June 14, 1978, 11.30 am			
Cryptomonas sp. - 15.12 µ	P	192	42
Rhodomonas pusilla	P	71	15
Gymnodinium - Peridinium - 15 µ	P	63	14
Skeletonema	K	39	8
Chaetoceros (large)	K	27	6
" (small)	K	23	5
Aphanizomenon flos-aquae	B	16	3
Peridinium minusculum	P	11	2
Oscillatoria limnetica	B	9	2
Pyramimonas	G	8	2
		459	99
KAGGFJAERDEN, June 14, 1978, 6.00 pm			
Skeletonema costatum	K	138	20
Cryptomonas sp.	P	112	16
Chaetoceros (small)	K	111	16
" (large)	K	110	16
Monads, 3-5 µ + Pseudopedinella	C	100	14
Rhodomonas pusilla	P	83	12
Pyramimonas	G	16	2
Gymnodinium - 15 - 20 µ	P	12	2
" - 10 - 15 µ	P	8	1
		690	99

/51