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Clarence A. Brown, Jr., Olin Jarrett, Jr., and Franklin H. Farmer
Langley Research Center
Hampton, Virginia

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INTRODUCTION

The concentration of chlorophyll a, the photosynthetic pigment found in plants, is used by biological oceanographers and limnologists as an indicator of the phytoplankton biomass of a body of water. Chlorophyll a is the key compound in the conversion of solar energy into living plant tissue. Chlorophyll a is found in the algae which form the base of a complex food chain in the marine environment. Zooplankton selectively graze certain types of algae (the golden-brown and green color groups), whereas the dominance of other types (blue-green color group) is often associated with pollution and low productivity. Assessment of the health of a body of water generally involves both determination of chlorophyll a concentrations and microscopic cell counts to identify species present.

Using water samples collected from a body of water, concentrations of chlorophyll a in living phytoplankton (in vivo) or extracted from phytoplankton cells (in vitro) are measured by a variety of spectroscopic and chemical techniques (Lorenzen, ref. 1; Strickland and Parsons, ref. 2; Yentsch and Menzel, ref. 3; and Holm-Hansen et al., ref. 4). Because of the large spatial variability in phytoplankton distributions (their so-called "patchiness"), numerous water samples must be collected at appropriate time and space intervals to describe adequately the chlorophyll a spatial distribution. The collection and subsequent analysis of these samples is time-consuming and costly and requires specially trained personnel.

Remote-sensing techniques are under development to determine chlorophyll a concentrations in vivo by measuring the fluorescence emitted by chlorophyll a when exposed to light. Laboratory studies (Lorenzen, ref. 1; and Yentsch and Menzel, ref. 3) have shown a correlation between the in vivo fluorescence produced and the concentration of chlorophyll a present. The light source can be the Sun, such as would be found in a passive remote system which senses the spectrum of upwelled light from the water column (e.g., Neville and Gower, ref. 5) or an active remote-sensing system using a laser operating at one or more wavelengths (Bristow et al., ref. 6; and Mumola and Kim, ref. 7).

NASA Langley Research Center has under development a remote, multiwavelength laser system (Mumola and Kim, ref. 7; Jarrett et al., ref. 8; Mumola et al., ref. 9; and Brown et al., ref. 10) designed to excite phytoplankton bearing chlorophyll a and measure the fluorescence generated by this excitation. In the Langley system, multiwavelength excitation takes advantage of the characteristic fluorescence excitation spectra of the four major algal color groups. The purpose of this system is to remotely identify and map the distribution of color groups as well as to determine the total chlorophyll a concentration. Earlier descriptions of the system may be found in references 9 and 10, and the theoretical basis of the technique for computation of chlorophyll a concentration (density) from fluorescence data has been reported by Mumola et al. in reference 9 and later by Browell in reference 11.

A series of tank tests were conducted in the laboratory in which a pure culture of algae from each of the four color groups was grown under controlled conditions of light, nutrients, and temperature. Remote measurements were made at intervals throughout the growth period with the Langley fluorosensor and compared with measurements made by conventional techniques. The purpose of these tests was to assess the validity of the theoretical model used to compute chlorophyll $\underline{a}$ concentrations from remote measurements of laser induced fluorescence and to test the ability of the Langley fluorosensor data to reveal the color group present. Results of these tests are presented herein.

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SYMBOLS

A   effective area of receiving telescope primary mirror (0.0380 m^2)

a   slope of regression equation

$B(\lambda_i)$ background fluorescence for excitation wavelength λ_i, mg/m

b   intercept of regression equation

C.V.   coefficient of variation, $\dfrac{\text{Standard deviation}}{\text{Mean}} \times 100$, percent

d   culture depth, m

$F(\lambda_i)$ fluorescence resulting from excitation at λ_i, mg/m

$F^*(\lambda_i)$ fluorescence $F(\lambda_i)$ corrected for the intercept, mg/m

K $= \left\{ \left[\dfrac{\xi A}{4\pi R^2 m^2} \left(\dfrac{\Delta\lambda_D}{\Delta\lambda_f}\right)\left(\dfrac{\theta_r}{\theta_L}\right)^2 \right] \left[1 - \dfrac{(mR)^2}{(mR + d)^2} e^{-(\alpha_f + \alpha_i)d} \right] \right\}^{-1}$

m   index of refraction of water (1.333)

n   chlorophyll $\underline{a}$ molecular density, molecules/m^3

PMT   photomultiplier tube

$P_o(\lambda_i)$ laser energy output at wavelength λ_i, J

$P_r(\lambda_f, \lambda_i)$ energy received by the sensor at wavelength λ_f after excitation of phytoplankton at wavelength λ_i, J

R   distance from laser to water (17.4 m)

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r	correlation coefficient
Va-9	VIMS designation for *Anacystis marina*
Va-12	VIMS designation for *Pseudoisochrysis paradoxa*
Va-13	VIMS designation for *Prorocentrum minimum*
Va-70	VIMS designation for *Porphyridium purpureum*
Va-72	VIMS designation for *Phaeodactylum tricornutum*
Va-74	VIMS designation for *Dunaliella euchlora*
VIMS	Virginia Institute of Marine Science
α_f	attenuation coefficient of water at 685 nm, m^{-1}
α_i	attenuation coefficient of water at excitation wavelength λ_i, m^{-1}
$\Delta\lambda_D$	spectral width of detector, nm
$\Delta\lambda_f$	spectral width of fluorescence, nm
θ_L	beam divergence of laser, sr
θ_r	receiver field of view, sr
λ_f	fluorescence wavelength (685 nm)
λ_i	excitation wavelength, nm
ξ	total optical efficiency (0.226)
$\sigma(\lambda_i)$	fluorescence cross section; the fluorescence energy emitted at λ_f = 685 nm after excitation at λ_i per molecule of chlorophyll *a*, divided by the incident energy per unit area, m^2/molecule

FLUOROSENSOR

The fluorosensor used to demonstrate the multiwavelength excitation concept of chlorophyll *a* detection in phytoplankton was designed and fabricated at Langley Research Center. A schematic of the system is presented in figure 1, and photographs are shown in figure 2. The fluorosensor is a unique four-color dye laser pumped by a single linear xenon lamp (invention by Mumola and McAlexander, ref. 12). The flash lamp was double processed by the manufacturer in an effort to increase the lamp life. This process required that the xenon gas be sealed in the flash lamp, then fired a number of times to assure that the gaseous impurities were suspended in the xenon gas. The lamp was evacuated,

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a new charge of xenon gas was injected into the flash lamp, and the lamp permanently sealed. The laser head, shown in cross section in figure 3, consists of elliptical cylinders spaced 90° apart with a common focal axis. The linear flash lamp is placed along this common focal axis, and its radiant energy is equally divided and focused into the dye cells located on the surrounding focal axis. The dye cells contain ethanol solutions of the fluorescent dyes, 7-diethylamino-4-methylcoumarin (4×10^{-4} M), coumarin 6 (3×10^{-4} M), rhodamine 6G (3×10^{-4}), and acridine red (4×10^{-4} M), which lase at 454, 539, 598, and 617 nm, respectively, and form the active medium for the four separate dye lasers. A rotating intracavity shutter permits only one wavelength at a time to be transmitted downward to the water. A photograph showing the laser head, cavity, and shutter mounted in the system is shown in figure 4.

A multilayer dielectric, low-pass optical filter was deposited on the window located between the laser and the telescope. The purpose of the filter is to block broadband dye fluorescence which occurs after lasing has been quenched. Broadband fluorescence backscattered from the culture tank cannot be distinguished from chlorophyll a fluorescence and thus must be blocked. The transmission of the filter is <1 percent between 640 and 720 nm and >85 percent at all laser wavelengths. The window is mounted at a 5° angle of incidence to the laser beams to prevent the reflected energy from returning to the laser output mirror. This prevents a false signal from entering the laser-energy monitoring system. The dyes and flash lamp cooling water are maintained at a uniform temperature by means of a small refrigerator and a submerged heating coil in the system. The energy for the flash lamp is provided by a high voltage supply, charging network, coaxial capacitor, generator, and spark gap. The output energies of the four lasers ranged from 2 to 4 millijoules (mJ) with a pulse duration of approximately 300 nanoseconds (ns). The full divergence angle of the beam is 5 milliradians (mrad).

After the laser energy has been transmitted to the phytoplankton, the resulting fluorescence of the algae is diffuse, and only a small portion is collected by the sensor telescope. The telescope is an f-16, 25.4-cm diameter, Dall-Kirkham type with a transmission of 74 percent. The field of view of the 25.4-cm telescope is 9 mrad, and the effective area of the telescope primary mirror is 0.0380 m^2. Light is concentrated by the telescope and passed through a 9-nm band-pass optical filter, with 36 percent maximum transmission centered at 685 nm. A 9-mm thick piece of RG 645 Schott Optical Glass (transmission of 92 percent at 685 nm) assures blocking of direct laser backscatter and much of the broadband fluorescence from the laser beam. The system uses two different detectors. One is a 12.7-mm diameter photodiode biased at 22 volts (to provide minimal rise time) to detect the energy output of each laser pulse; the other is a photomultiplier to detect fluorescence from chlorophyll a. The photodiode is optically linked by a bundle of fiber optics to the perimeter of each laser output mirror as shown by Jarrett and Northam in reference 13. The photomultiplier is a 45.7-mm diameter, end-on type photocathode photomultiplier tube (PMT), RCA 8852, with 12 dynodes operating at 1500 volts (V).

Current output of the PMT in response to the laser-induced signal is typically larger than the current flow through the voltage divider chain in the PMT base. Capacitors are provided between the last five PMT stages to store current for such pulsed operation. The PMT is normally gated (switched off) to

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allow charge buildup on the capacitors. Gating is achieved by setting the potential of the fourth dynode below that of the third, then applying a capac- itatively coupled positive pulse during data collection to turn on the PMT. Output current signals from the PMT are integrated, digitized, displayed, and recorded on magnetic tape.

Direct calibration of the laser-energy monitoring system was provided by periodically measuring the laser output with an energy meter whose characteris- tics have been compared with specifications of the National Bureau of Stan- dards. The photomultiplier output current sensitivity at 685 nm and 1500 V was provided by the manufacturer. This output remained constant when compared with an identical tube used for calibration checks. No noticeable temperature drift was observed from the photomultiplier outputs when used in the fluorosensor operation mode.

During the early phase of testing, control of the data events was achieved by a master pulse generator which sequentially gated the photomultiplier tube, initiated integration, fired the laser, and triggered a monitoring dual beam scope. In the latter part of the test phase, a microprocessor assumed these command functions.

LABORATORY TESTS

A schematic of the laboratory apparatus used for this study of remote measurements of fluorescence from chlorophyll a in phytoplankton is shown in figure 5. The phytoplankton cultures were grown in a disinfected tank, 45.72 cm × 45.72 cm × 45.72 cm, with a volume of approximately 100 ℓ. To avoid contamination, the tank was coated with black silicone rubber, which pre- vented the sea water medium from contacting the metal sides. This coating also minimized optical wall and bottom effects. The culture tank was immersed in a controlled temperature water bath at 19°C and continuously illuminated with six commercially available fluorescent "grow" lights. During growing cycles, these lights were located about 8 cm above the tank. The tank was filled with natural sea water (>30 $^{\circ}/_{\circ\circ}$ salinity) obtained from the Atlantic Ocean near Wachapreague Island in Virginia, centrifuged, heat sterilized, and stored until needed. A nutrient supplement containing sodium nitrate, sodium phosphate, sodium metasilicate, vitamins, iron-ethylenediamine-tetraacetic acid solution, and micronutrients was added to the sea water to insure growth of the phyto- plankton. The ingredients for this medium are given in table I. To facilitate the growth cycle, gentle agitation of the medium was provided by a plastic pro- peller turning at 15 cycles per minute. To assure that the organisms were grown without stress, the nitrate level was maintained at approximately 10 mg/ℓ through the test period.

The laser beams from the fluorosensor were directed through turning mirrors to a mirror located on the ceiling and then into the tank at an incidence angle nearly perpendicular to the surface. The fluorescence signal returned by the same path. The range (total path length) from the detector to the water surface was 17.4 m. After all of the fluorosensor data were collected, the position of the image of the culture tank formed by the telescope was calculated to be 1.24 m behind the field-of-view-defining aperture located at the focal plane of

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the telescope. A calculation was made to determine the area containing the defocused image at the telescope focal plane. This calculation showed that, for a range of 17.4 m, the field-of-view-defining aperture admitted only 22.8 percent of the available energy; therefore, the data collected were modified to account for this loss of energy. The footprint of the laser beam at the surface of the water was 8.7 cm, and the telescope field of view was 41.06 cm in diameter.

Fluorosensor measurements were made once or twice a day, depending on the growth rate of the organism, for a period of 1 week to 10 days. Initial inoculation of the sea water medium produced a cell count of 10^2 to 10^3/ml. At the time of each test, a water sample was siphoned for laboratory analysis of chlorophyll a concentration, in vivo fluorescence of chlorophyll a, cell count, and effective light attenuation coefficients.

Effective attenuation coefficients were established in a two-step process using part of the water sampled from the tank. First, a helium-neon laser, shown in figure 6, was used to measure the amount of light (at 632.8 nm) transmitted through glass tubes of different lengths containing water from the tank. The method of Duntley (ref. 14) was used to calculate the attenuation coefficient of light at 632.8 nm. This apparatus had a total collection angle of 4.86°. This collection angle was sufficiently large so that beam attenuation could not be measured and small enough that diffused attenuation could not be measured, resulting in an effective attenuation coefficient somewhere between the two. This effective attenuation coefficient was selected for the required values of the attenuation coefficients, an approach similar to that of Gordon (ref. 15) and McCluney (ref. 16). Second, a sample was scanned using a Cary[1] 17 transmission spectrophotometer in the absorbance mode with distilled water in the reference cell. This provided an attenuation spectrum relative to distilled water. The absorbance of distilled water was added to the attenuation spectrum, and the attenuation spectrum was normalized to the value of the effective attenuation coefficient at 632.8 nm, determined previously, to give a calibrated spectrum of attenuation coefficients for that sample. Three examples of effective attenuation coefficient spectra of algal cultures are shown in figure 7.

PHYTOPLANKTON CHARACTERISTICS

Six different species of phytoplankton were tested. Each species was grown at least twice in a pure culture beginning with an initial inoculation concentration of about 1 mg/ℓ chlorophyll a and ending with chlorophyll a concentrations generally in excess of 50 µg/ℓ. The species were selected on the basis of availability, ease of growth, and color group, with at least one from each of the four major color groups. The species of phytoplankton used in the study are summarized in table II. The "Va" nomenclature is a Virginia Institute of Marine Science culture designation. Characteristics of the phytoplankton are described next.

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[1]Cary: Registered trade name of Varian.

Anacystis marina (Va-9)

This single-cell blue-green marine alga is approximately 1 to 2 μm in diameter. The major pigments are chlorophyll a and the phycobilin phycocyanin. The blue-green color is a result of the phycocyanin, which has distinctive absorbance and fluorescence spectra. Anacystis marina tends to grow rapidly in culture but is unpredictable and may at times grow very slowly or not at all.

Pseudoisochrysis paradoxa (Va-12)

This golden-brown alga is a small, motile, marine alga placed in the Class Chrysophyceae. This species contains chlorophylls a and c, as well as the carotenoid fucoxanthin. This species generally tends to grow slowly in cultures.

Prorocentrum minimum (Va-13)

Prorocentrum minimum is a golden-brown, marine, armored dinoflagellate with the cell wall possessing two valves. The two flagella are located anteriorly. This organism is known to tolerate a wide range of temperature and salinity. In cultures, it grows slowly but steadily. Pigments include chlorophylls a and c, and peridinin, the primary carotenoid of the dinoflagellates. This particular organism is often the dominant phytoplankton in the lower York River in Virginia.

Porphyridium purpureum (Va-70)

Porphyridium purpureum is a nonmotile, round, marine, red alga. The deep red color is imparted by the phycobilin pigment, phycoerythrin. This phycobilin occurs primarily in rhodophytes, cyanophytes, and cryptophytes. This organism also contains the carotenoid pigment lutein and exhibits a moderate growth rate in cultures.

Phaeodactylum tricornutum (Va-72)

Phaeodactylum tricornutum is a marine, golden-brown diatom with a distinctive three-point form, although individuals with only two points are frequently observed. The pigment fucoxanthin, typical of diatoms though not restricted to them, is the major carotenoid. Chlorophylls a and c are also present. This organism is the most rapid growing of the species studied.

Dunaliella euchlora (Va-74)

Dunaliella euchlora is a motile, marine, green alga. It carries the chlorophylls a and b combination typical of the chlorophytes and euglenophytes, and carotenoid pigment lutein. This algal division is closely related to higher terrestrial plants. Dunaliella euchlora exhibits a steady, though not rapid, growth in cultures.

THEORETICAL MODEL

The fluorescence energy received by the sensor at wavelength λ_f after excitation of phytoplankton by laser energy at wavelength λ_i is described by the mathematical model shown in figure 8. This model illustrates a specific case of the derivation in reference 11. The model assumes that a narrow beam of laser light is transmitted through the atmosphere and water according to Beer's law. At the air-water interface, a small portion of the laser beam is reflected back into the atmosphere, and the remainder refracted. The refracted beam is then transmitted through the water column, where the attenuation coefficient is α_i.

The model assumes that the chlorophyll a molecular density is constant over the water column or, more specifically, over that portion penetrated by the laser light. In addition, it is assumed that there is no fluorescence contribution from other materials. Some of the light incident on the algae is absorbed and a portion transferred to chlorophyll a pigments, where it may be used for photosynthesis. Excess light energy not used for photosynthesis or converted to heat is emitted (fluoresced), with the peak fluorescence being at $\lambda_f = 685$ nm. Fluorescence is assumed to be emitted uniformly in all directions (i.e., isotropically) and, therefore, only a small fraction of the total laser-induced fluorescence is captured by the fluorosensor. Diffuse fluorescence at wavelength λ_f is transmitted upward through the water column, where the appropriate attenuation coefficient is now α_f. Again, after some internal reflection, the remaining light is refracted at the air-water interface before being transmitted to the sensor. At the sensor, a filter transmits only light in a 9-nm-wide band centered at 685 nm.

While details concerning derivation of the mathematical model (fig. 8) may be found in Browell (ref. 11), several modifications and simplifications were made for the purposes of this study. Atmospheric attenuation and surface reflectance have been assumed negligible. The finite depth term was derived by changing the depth limits of integration from 0 to ∞, to 0 to d (culture depth) and proceeding as in reference 11. It is assumed that light reaching the bottom or sides of the tank is totally absorbed by the black walls.

An important model parameter is the fluorescence excitation cross section $\sigma(\lambda_i)$, which is a measure of the fluorescence efficiency of the chlorophyll a molecule. The fluorescence excitation cross section is defined as the fluorescence energy emitted at $\lambda_f = 685$ nm after excitation at λ_i per molecule of chlorophyll a, divided by the incident energy per unit area. The units of the cross section are square meters per molecule.

A study to determine fluorescence excitation cross sections for about 50 different marine and fresh-water algae, including those of this study, was conducted with the cooperation of the Virginia Institute of Marine Science (VIMS) using a fluorescence spectrophotometer technique described in reference 8. The resultant fluorescence excitation cross section spectra representative of the four major color groups are shown in figure 9 and listed in table III.

The spectral characteristics of the fluorescence excitation cross sections of the four major algae color groups are significantly different. This is pri-

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marily because the various pigments which characterize the color groups absorb exciting energy differently and transfer this energy to chlorophyll a with varying efficiencies. This spectral difference in fluorescence excitation cross sections is the basis for the remote fluorosensor's ability to indicate phytoplankton composition, that is, to classify the algae color group, as well as quantify the chlorophyll a concentration.

RESULTS AND DISCUSSION

Laboratory Versus Remote Chlorophyll a Data

The fluorosensor equation (fig. 8) was solved for n, the chlorophyll a molecular density. This remote chlorophyll a value was compared with estimates of chlorophyll a concentrations based on a standard laboratory analysis prescribed by Strickland and Parsons (ref. 2). With the exception of the attenuation coefficients and water depth, which were measured at the time of each fluorosensor test, all model parameters in the fluorosensor equation were measured in advance of the tests and assumed to remain constant thereafter. The cross sections used were those derived with the fluorescence spectrophotometer (table III).

Both types of chlorophyll a concentration estimates – laboratory and remote – are plotted against time in figures 10 to 15 to show the comparisons at various stages of growth. In general, the four remote chlorophyll a estimates, corresponding to the four lasers, agree well among themselves. The remote estimates agree generally within a factor of 3 with extracted chlorophyll a estimates.

The golden-brown species included Pseudoisochrysis paradoxa (Va-12), grown three times (fig. 10); Prorocentrum minimum (Va-13), grown three times (fig. 11); and Phaeodactylum tricornutum (Va-72), grown four times (fig. 12). There was a consistent tendency for the remote estimates to be lower than the extracted chlorophyll a estimates, although the two estimates showed similar growth patterns for each species.

The green species, Dunaliella euchlora (Va-74) was grown four times (fig. 13). Good agreement was observed between the remote chlorophyll a estimates and the extracted laboratory estimates.

The red alga, Porphyridium purpureum (Va-70), was grown twice. This organism experienced a moderate growth rate with approximately 3.5 doublings in 5 days (fig. 14(a)) and 6 doublings in 10 days (fig. 14(b)). In these cultures, the green (539 nm), orange (598 nm), and red (617 nm) lasers generally gave mutually consistent results, but the remote chlorophyll estimate based on the blue (454 nm) laser was significantly higher than the others. With the exception of the blue laser, agreement between the remote chlorophyll a values and extracted chlorophyll a values was good.

The most erratic results were those of the blue-green alga, Anacystis marina (Va-9), which are shown in figure 15. This organism presented a number of problems during attempts to grow it in the 100-ℓ tank. This is evidenced by

the growth patterns shown in figure 15. Three attempts were made to grow Va-9. The first resulted in the organism not growing at all, and fluorescence values could not be obtained with the fluorosensor. The organism is small in size, and several attempts to obtain it in sufficient quantity to be used in the tank test were unsuccessful. Cultures of Va-9 could be grown in glass flasks, but when an attempt was made to inoculate the water medium in the 100-ℓ tank, difficulty was experienced in establishing a growth pattern. Several factors may have contributed to the poor growth patterns experienced by the Va-9 phytoplankton: (1) possible stress conditions when the tank was inoculated with culture, (2) bacteria, being of similar size as Va-9 phytoplankton, growing faster than the phytoplankton, thereby inhibiting a good growth rate, and (3) poor cultures of Va-9 used to inoculate the tank. The exact reason for the poor growth of Va-9 is not known.

Only on the second tank test, February 25 to March 4 (fig. 15a)), did the algae establish an early positive growth rate. In fact, it was necessary to dilute the sample on March 2 because the culture became dense, and the chlorophyll a content exceeded that which would normally be expected in the natural environment. The blue laser (454 nm) gave higher results than did the other three lasers. These three lasers gave mutually consistent results but the remote estimates were greater than the laboratory estimates for chlorophyll a.

On the third tank test, figure 15(b), (April 25 to May 3, 1977), the green (539 nm), orange (598 nm), and red (617 nm) lasers showed values for remote chlorophyll a similar to the extracted values. Again, the blue laser (454 nm) overestimated the remote chlorophyll a throughout the growth.

Based on the evidence shown in figures 10 to 15, it is concluded that the fluorescence measured by the fluorosensor provides good quantitative measures of chlorophyll a concentrations for all species and lasers except the blue laser estimates for Va-9 and Va-70, when compared with the laboratory extraction technique. Although there is good agreement between fluorosensor and extracted chlorophyll a values in these studies, it is recognized that phytoplankton are living organisms and subject to variance. Other possible reasons for disagreement are (1) the fluorosensor mathematical model may not be properly formulated, (2) the energy values measured by the fluorosensor are subject to error, and (3) the cross sections developed in reference 8 may have been inaccurate in part because of the growth conditions. This may require that data obtained by the fluorosensor be adjusted using "in situ" chlorophyll a measurements to determine cross sections for a particular test or environment.

Table IV lists linear regression coefficients and correlation coefficients for regressions of the laboratory chlorophyll values versus each of the four fluorosensor estimates of chlorophyll a. Some of the high correlation values are influenced by the single values of chlorophyll a at the end of the growth phase for some of the species tested. Even though these high values may influence the correlation coefficients, the use of the high values (in all cases except Va-9) can be shown to be appropriate in forming the linear regressions since the cultures are in log phase growth.

Behavior of Fluorescence Excitation Cross Sections

The cross section is a measure of how efficient the phytoplankton is in converting excitation energy to fluorescence energy. It is analogous to the fluorescence-to-chlorophyll ratio, which is known to vary considerably in other in vivo fluorometric methods such as with the Turner Model III fluorometer (ref. 1). Solving the fluorosensor equation for $\sigma(\lambda_i)$ yields

$$\sigma(\lambda_i) = \frac{F(\lambda_i)}{n} \tag{1}$$

where, to be consistent with the above analogy, the term $F(\lambda_i)$ is called the fluorescence and is given by the equation

$$F(\lambda_i) = \frac{K(\alpha_i + \alpha_f) P_r(\lambda_i, \lambda_f)}{P_o(\lambda_i)} \tag{2}$$

where K is the reciprocal of the product of the first and last bracketed terms in figure 8.

For each test, four fluorescences corresponding to the four lasers were calculated and plotted against the laboratory chlorophyll a concentrations. In figures 16 to 21 the fluorescence in terms of molecules per meter is converted to the units of mass per meter by use of the factor 1.498×10^{-18} mg/molecule. Linear regressions were calculated, and the slopes of these regressions were assumed to be the appropriate cross sections for the fluorosensor tests. These plots and regression lines are shown in figures 16 to 21 and are listed in table V. It should be noted that in figures 16 to 21 there is a good linear fit between the fluorosensor fluorescence and extracted chlorophyll a for all species except for one test involving the blue-green algae Va-9 (fig. 21). As previously noted, this organism was difficult to grow and did not sustain log phase growth throughout any single test. Table V lists linear regression and correlation coefficients for each of the four fluorosensor measurements of fluorescence versus extracted chlorophyll a densities. As in table IV, high correlation values were strongly influenced by single values of chlorophyll a at the end of some of the tests. However, the use of these high values is appropriate in forming the linear regressions since the cultures (except as noted) are in log phase growth.

Strictly speaking, the mathematical model shown in figure 8 would imply that the linear regression of $F(\lambda_i)$ against n should pass through the origin (i.e., $F(\lambda_i) = 0$ when $n = 0$). An intercept term was found for the regressions shown in figures 16 to 21. If the intercept is other than zero, it could possibly be due to errors in the recorded data or in the form of the mathemati-

cal model used. No attempt was made to determine the physical or statistical significance of the intercepts.

Averages of the fluorescence cross sections (slopes) derived from the fluorosensor tests for each species are listed in table VI. It was of interest to compare these cross sections with those in table III that were derived using a fluorescence spectrophotometer and the technique of reference 8. It was hypothesized that these fluorescence cross sections differ only in magnitude, not in spectral shape. Figure 22 shows plots of the fluorescence cross section spectra (solid lines) from the spectrophotometer study compared with the fluorescence cross sections derived in the fluorosensor tests (circles). The dashed curves are spectra, as hypothesized, that retain the same shape. The error bars indicate the observed data range.

Constancy of shape is equivalent to constancy in the ratio of fluorescence cross sections at two different excitation wavelengths λ_i and λ_i', (i.e., $\sigma(\lambda_i)/\sigma(\lambda_i') = $ Constant). If the fluorescence is modeled by the linear equation

$$F(\lambda_i) = \sigma(\lambda_i)n + B(\lambda_i) \tag{3}$$

where $B(\lambda_i)$ is the intercept, then

$$\frac{F(\lambda_i) - B(\lambda_i)}{F(\lambda_i') - B(\lambda_i')} = \frac{\sigma(\lambda_i)}{\sigma(\lambda_i')} = \text{Constant} \tag{4}$$

Letting $F^*(\lambda_i)$ denote the fluorescence corrected for the intercept (i.e., $F^*(\lambda_i)$ would be the chlorophyll a fluorescence if $B(\lambda_i)$ is a background fluorescence), then the following ratios were computed from the results obtained on a daily basis as the cultures grew:

$$\frac{F^*(539)}{F^*(454)} \qquad \frac{F^*(598)}{F^*(539)} \qquad \frac{F^*(617)}{F^*(539)}$$

Means, standard deviations, and coefficients of variation for these ratios are given in table VII. With the exception of the second test of the blue-green species (Va-9), the coefficients of variation were generally less than 30 percent and, in more than half of the cases, they were less than 10 percent. Fluorescence ratios computed from unpublished data previously collected by the authors and measured by the method of reference 8 are shown in table VIII. Comparisons of tables VII and VIII show similar values for the fluorescence ratio, thereby supporting the hypothesis that the shape of a fluorescence-cross-section curve remains constant.

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On the basis of these results shown in table VII, it is concluded that differentiation among the red, golden-brown, and green color groups may be achieved by inspection of fluorescence ratios. For example, the ratio $F^*(539)/F^*(454)$ is between 0.6 and 1.0 for golden-brown species, approximately 0.3 for greens, approximately 4.0 for reds, and approximately 3.0 for blue-greens. Differentiation among the golden-browns (e.g., diatoms versus dinoflagellates) appears unlikely based on these data. Other ratios show similar differences between the various species. These differences in fluorescence-cross-section ratios are the basis for determining composition of phytoplankton population according to color group when a multiwavelength source of excitation is used.

CONCLUDING REMARKS

A series of tests were performed in the laboratory to test the ability of a remote laser fluorosensor, developed at the Langley Research Center, to measure the concentration of chlorophyll a in tanks containing pure cultures of phytoplankton. Six different phytoplankton species were tested; each was grown two to four different times. The fluorosensor uses a unique four-color dye laser system pumped by a single linear xenon lamp to induce fluorescence in chlorophyll a molecules contained in phytoplankton.

The following results were shown in data from these tests:

(1) The fluorescence measured by the fluorosensor provides good quantitative measurement of chlorophyll a concentrations for all species tested while the cultures were in log phase growth (except for tests with the blue laser on Va-9 and Va-70).

(2) Fluorescence cross section ratios obtained in the single species tank tests support the hypothesis that the shape of the fluorescence-cross-section curve remains constant with species. Differences in fluorescence-cross-section ratios are a basis for determining diversity of phytoplankton according to color group when a multiwavelength source of excitation is used.

(3) Linear relationships exist between extracted chlorophyll a concentration and fluorescence measured by the remote fluorosensor during the log phase growth of phytoplankton cultures tested.

Langley Research Center
National Aeronautics and Space Administration
Hampton, VA 23665
February 20, 1981

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TABLE I.- SEA WATER MEDIUM USED IN LABORATORY TESTS

The medium used to grow the algal species was made from sea water at >30 $^o/_{oo}$ and enriched as follows. (Solutions (1) to (6) were autoclaved after preparation):

(1) Sodium nitrate solution
    NaNO$_3$ . . . . 1.0 g
    Distilled water . . . . 1.0 ℓ

(2) Sodium phosphate solution
    Na$_2$HPO$_4$·7H$_2$O . . . . 2.0 g
    Distilled water . . . . 1.0 ℓ

(3) Micronutrients solutions
    (a) FeSO$_4$·7H$_2$O . . . . 4.98 g
        ZnSO$_4$·7H$_2$O . . . . 8.82 g
        MnCl$_2$·4H$_2$O . . . . 1.44 g
        Distilled water . . . . 1.0 ℓ
    (b) MoO$_3$ . . . . 0.71 g
        Co(NO$_3$)$_2$·6H$_2$O . . . . 0.41 g
        Distilled water . . . . 1.0 ℓ
    (c) Ethylenediamine-tetraacetic acid (EDIA) . . . . 50 g
        KOH . . . . 31 g
        Distilled water . . . . 1.0 ℓ
    (d) H$_3$BO$_3$ . . . . 11.42 g
        Distilled water . . . . 1.0 ℓ

(4) Iron-EDTA solution
    Fe(NH$_4$)$_2$(SO$_4$)$_2$·6H$_2$O . . . . 7.02 g
    NA$_2$EDTA . . . . 6.60 g
    Distilled water . . . . 1.0 ℓ

(5) Vitamin stock solution
    Biotin . . . . 0.1 mg
    B$_{12}$ . . . . 0.1 mg
    Thiamin HCl . . . . 20.0 mg
    Distilled water . . . . 100 ml

(6) Sodium metasilicate solution
    Na$_2$SiO$_3$·9H$_2$O . . . . 4.66 g
    Distilled water . . . . 100 ml

The above enrichments were added to 100 ℓ of filtered sea water as follows.

    Sodium nitrate solution . . . . 1 ℓ
    Sodium phosphate solution . . . . 1 ℓ
    Micronutrient solution (a) . . . . 100 ml
    Micronutrient solution (b) . . . . 100 ml
    Micronutrient solution (c) . . . . 100 ml
    Micronutrient solution (d) . . . . 100 ml
    Iron EDTA solution . . . . 100 ml
    Vitamin stock solution . . . . 100 ml
    Sodium silicate solution . . . . 100 ml

TABLE II.- PHYTOPLANKTON USED IN LABORATORY TESTS

Color group	Identification	Division	Class	Order	Genus and species
Blue-green	Va-9	Cyanophyta	Cyanophyceae	Chroococcales	Anacystis marina
Golden-brown (yellow-green)	Va-12	Chrysophyta	Chrysophyceae	--------------	Pseudoisochrysis paradoxa
Golden-brown (dinoflagellate)	Va-13	Pyrrophyta	Dinophyceae	Prorocentrales	Prorocentrum minimum
Red	Va-70	Rhodophyta	Rhodophyceae	Porphyridiales	Porphyridium purpureum
Golden-brown (diatom)	Va-72	Chrysophyta	Bacillariophyceae	Bacillariales	Phaeodactylum tricornutum
Green	Va-74	Chlorophyta	Chlorophyceae	Volvocales	Dunaliella euchlora

TABLE III.- FLUORESCENCE CROSS SECTIONS BY COLOR GROUPS USING

TECHNIQUE OF REFERENCE 8

Organism	Fluorescence cross section, m^2/molecule			
	454 nm	539 nm	598 nm	617 nm
Va-9 (blue-green)	7.55 E-23	2.18 E-22	1.05 E-21	1.73 E-21
Va-12 (golden-brown)	7.58 E-22	4.57 E-22	3.39 E-22	5.11 E-22
Va-13 (dinoflagellate)	2.94 E-21	1.95 E-21	1.03 E-21	1.31 E-21
Va-72 (diatom)	8.91 E-22	6.37 E-22	2.99 E-22	4.35 E-22
Va-74 (green)	3.46 E-22	9.86 E-23	2.47 E-22	3.04 E-22
Va-70 (red)	4.48 E-23	3.99 E-22	1.09 E-22	1.35 E-22

TABLE IV.- LINEAR REGRESSION AND CORRELATION COEFFICIENTS FOR LABORATORY CHLOROPHYLL a WITH

FLUOROSENSOR CHLOROPHYLL a ESTIMATES

Organism	Date	454 nm			539 nm			598 nm			617 nm		
		a	b	r	a	b	r	a	b	r	a	b	r
Va-9 (blue-green)	2/25/77 - 3/4/77	.327	-11.038	.7012	.434	-6.280	.9399	.401	.618	.9788	.398	-2.259	.9724
	4/25/77 - 5/3/77	.599	-1.590	.9724	.616	.850	.8078	.158	1.980	.3805	.211	1.950	.3352
Va-12 (golden-brown)	7/26/76 - 7/30/76	1.375	2.980	.9922	1.315	3.354	.9813	1.390	5.410	.9774	1.943	2.020	.9939
	8/2/76 - 8/9/76	1.471	-.091	.9991	1.243	-.337	.9979	1.227	.250	.9959	.992	-.267	.9983
	8/9/76 - 8/16/76	1.622	.099	.9996	1.201	.415	.9985	1.801	.095	.9987	1.783	.787	.9981
Va-13 (dinoflagellate)	7/9/76 - 7/19/76	3.037	-1.302	.9973	2.688	.548	.9918	2.972	-.247	.9945	3.265	-.167	.9928
	8/27/76 - 9/7/76	2.048	-1.621	.9984	2.158	-2.257	.9978	2.643	-3.042	.9975	3.049	-3.004	.9976
	9/13/76 - 9/20/76	2.140	-2.654	.9939	1.987	-1.809	.9970	2.289	-1.772	.9974	2.836	-2.265	.9956
Va-70 (red)	11/17/76 - 11/23/76	.645	-16.490	.9847	1.206	-2.420	.9861	.995	-7.650	.9900	1.007	-12.840	.9728
	3/28/77 - 4/7/77	.405	-21.350	.9918	.702	-6.477	.9950	.575	-9.529	.9962	.632	-15.300	.9706
Va-72 (diatom)	10/5/76 - 10/18/76	1.898	.305	.9935	1.612	-.168	.9963	2.178	-2.070	.9992	2.311	-3.030	.9987
	10/12/76 - 10/19/76	1.497	.283	.9996	1.025	1.860	.9999	1.307	-1.500	.9997	1.389	-3.300	.9997
	1/28/77 - 2/3/77	1.828	15.619	.9710	1.682	16.805	.9671	1.602	17.200	.9636	1.604	4.394	.9738
	6/28/77 - 7/2/77	4.013	-2.437	.9808	2.998	-.301	.9874	3.093	-4.509	.9901	2.554	-7.959	.9910
Va-74 (green)	9/20/76 - 9/27/76	2.156	-4.720	.9923	1.789	-11.830	.9792	2.721	-3.730	.9925	2.501	-2.850	.9928
	9/27/76 - 10/4/76	.876	-.190	.9985	.817	-4.520	.9987	1.098	.180	.9981	1.153	.061	.9985
	11/1/76 - 11/6/76	1.363	1.130	.9920	1.606	-6.950	.9881	1.899	.101	.9990	1.609	-.720	.9782
	6/22/77 - 6/27/77	1.116	10.490	.9970	.943	27.090	.9865	.988	25.248	.9885	.959	18.950	.9918

a = Slope

b = Intercept

r = Correlation coefficient

TABLE V.- LINEAR REGRESSION AND CORRELATION COEFFICIENTS FOR FLUORESCENCE WITH LABORATORY CHLOROPHYLL a ESTIMATES

Organism	Date	454 nm			539 nm			598 nm			617 nm		
		a	b	r	a	b	r	a	b	r	a	b	r
Va-9 (blue-green)	2/25/77 - 3/4/77	11.34 E-23	37.39 E-22	.7012	4.44 E-22	37.48 E-22	.9399	25.07 E-22	-5.04 E-22	.9788	41.08 E-22	12.23 E-21	.9724
	4/25/77 - 5/3/77	11.89 E-23	21.67 E-23	.9724	23.12 E-23	-6.75 E-24	.8078	9.65 E-22	39.50 E-23	.3805	9.26 E-22	14.31 E-22	.3352
Va-12 (golden-brown)	7/26/76 - 7/30/76	5.47 E-22	-15.71 E-22	.9922	33.47 E-23	-6.83 E-22	.9813	23.65 E-23	-11.93 E-22	.9774	26.12 E-23	-5.52 E-23	.9939
	8/2/76 - 8/9/76	5.14 E-22	4.52 E-23	.9991	36.66 E-23	13.35 E-23	.9979	27.41 E-23	-5.42 E-23	.9959	26.00 E-23	7.58 E-23	.9983
	8/9/76 - 8/16/76	4.67 E-22	-4.41 E-23	.9996	37.91 E-23	-14.73 E-23	.9985	18.79 E-23	-12.25 E-24	.9987	28.54 E-23	-21.19 E-23	.9981
Va-13 (dinoflagellate)	7/9/76 - 7/19/76	9.63 E-22	13.19 E-22	.9973	7.14 E-22	-31.19 E-23	.9918	34.27 E-23	12.88 E-23	.9945	39.54 E-23	13.27 E-23	.9928
	8/27/76 - 9/7/76	14.31 E-22	23.52 E-22	.9984	9.00 E-22	20.61 E-22	.9978	38.77 E-23	11.94 E-22	.9975	42.76 E-23	13.01 E-22	.9976
	9/13/76 - 9/20/76	13.57 E-22	38.60 E-22	.9939	9.76 E-22	18.51 E-22	.9970	4.47 E-22	8.28 E-22	.9974	4.58 E-22	10.98 E-22	.9956
Va-70 (red)	11/17/76 - 11/23/76	6.72 E-23	12.01 E-22	.9847	32.18 E-23	10.57 E-22	.9861	10.72 E-23	8.98 E-22	.9900	12.69 E-23	19.21 E-22	.9728
	3/28/77 - 4/7/77	10.88 E-23	24.06 E-22	.9918	5.63 E-22	38.19 E-22	.9950	18.82 E-23	18.41 E-22	.9962	19.90 E-23	34.40 E-22	.9706
Va-72 (diatom)	10/5/76 - 10/18/76	4.63 E-22	-38.24 E-24	.9935	39.23 E-23	11.68 E-23	.9963	13.71 E-23	28.83 E-23	.9992	18.78 E-23	5.78 E-22	.9987
	10/12/76 - 10/19/76	5.94 E-22	-15.03 E-23	.9996	6.21 E-22	-9.90 E-22	.9999	25.42 E-23	4.88 E-23	.9997	31.28 E-23	10.40 E-22	.9997
	1/28/77 - 2/3/77	4.59 E-22	-5.13 E-21	.9710	35.42 E-23	-41.87 E-22	.9671	17.32 E-23	-20.23 E-22	.9636	25.73 E-23	5.30 E-23	.9738
	6/28/77 - 7/2/77	21.34 E-23	6.55 E-22	.9808	20.72 E-23	13.32 E-23	.9874	9.47 E-23	4.61 E-22	.9901	16.58 E-23	13.83 E-22	.9910
Va-74 (green)	9/20/76 - 9/27/76	15.80 E-23	7.73 E-22	.9923	20.72 E-23	6.67 E-22	.9792	8.94 E-23	34.78 E-23	.9925	11.99 E-23	35.80 E-23	.9928
	9/27/76 - 10/4/76	39.47 E-23	9.03 E-23	.9985	5.28 E-23	5.52 E-22	.9987	22.41 E-23	-23.24 E-24	.9981	26.28 E-23	-4.47 E-24	.9985
	11/1/76 - 11/6/76	24.98 E-23	-22.49 E-23	.9920	6.00 E-23	4.47 E-22	.9881	12.98 E-23	-9.21 E-23	.9990	18.09 E-23	26.14 E-23	.9782
	6/22/77 - 6/27/77	30.81 E-23	-30.66 E-22	.9970	10.17 E-23	-25.56 E-22	.9865	24.41 E-23	-5.74 E-21	.9885	31.18 E-23	-5.50 E-21	.9918

a = Slope ≡ fluorescence cross section, σ

b = Intercept

r = Correlation coefficient

TABLE VI.- AVERAGES OF FLUORESCENCE CROSS SECTIONS BY COLOR GROUPS

FOR SINGLE SPECIES TESTS

Organism	Fluorescence cross section, m^2 per molecule, at -			
	454 nm	539 nm	598 nm	617 nm
Va-9 (blue-green)	1.1616 E-22	3.3741 E-22	1.7357 E-21	2.5184 E-21
Va-12 (golden-brown)	5.0936 E-22	3.6013 E-22	2.3283 E-22	2.6884 E-22
Va-13 (dinoflagellate)	1.2503 E-21	8.6307 E-22	3.9260 E-22	4.2699 E-22
Va-72 (diatom)	4.3267 E-22	3.9369 E-22	1.6479 E-22	2.3091 E-22
Va-74 (green)	2.7749 E-22	8.3773 E-23	1.7187 E-22	2.1894 E-22
Va-70 (red)	8.8012 E-23	4.4224 E-22	1.4805 E-22	1.6296 E-22

TABLE VII.- MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIATION FOR FLUORESCENCE RATIOS

Species	Date of lab test	$\dfrac{F^*(539)}{F^*(454)}$			$\dfrac{F^*(598)}{F^*(539)}$			$\dfrac{F^*(617)}{F^*(539)}$		
		Mean	Standard deviation	% C.V.[1]	Mean	Standard deviation	% C.V.	Mean	Standard deviation	% C.V.
Va-9 (blue-green)	2/28/77	4.030	1.128	27.99	5.694	.263	4.62	9.315	.562	6.03
	4/25/77	2.036	.837	41.11	4.143	2.030	48.99	3.603	2.971	82.45
Va-12 (YB-GB)	7/27/76	.609	.039	6.39	.736	.091	12.36	.791	.065	8.26
	8/02/76	.746	.098	13.10	.835	.239	28.66	.704	.053	7.54
	8/09/76	.905	.160	17.67	.487	.025	5.10	.771	.041	5.38
Va-13 (dinoflagellate)	7/12/76	.776	.067	8.63	.471	.021	4.39	.544	.026	4.72
	8/18/76	.662	.117	17.65	.425	.049	11.71	.493	.070	14.17
	9/13/76	.728	.063	8.69	.462	.031	6.81	.464	.033	7.09
Va-70 (red)	11/18/76	4.658	.766	16.45	.354	.042	11.93	.456	.092	20.11
	3/29/77	4.595	1.430	31.12	.371	.059	16.08	.366	.081	22.27
Va-72 (diatom)	10/05/76	.833	.036	4.37	.343	.024	6.93	.473	.033	7.00
	10/21/76	1.009	.101	10.04	.414	.015	3.56	.524	.018	3.56
	1/31/77	.773	.021	2.67	.489	.012	2.49	.724	.092	12.72
	6/28/77	.972	.130	13.42	.477	.038	8.06	.824	.050	6.11
Va-74 (green)	9/21/76	.369	.062	16.96	1.552	.237	15.29	2.065	.368	17.83
	9/27/76	.333	.031	9.37	1.713	.163	9.52	2.026	.161	7.96
	11/01/76	.249	.084	33.63	2.161	.461	21.33	2.906	1.099	37.82
	6/22/77	.358	.079	22.09	2.384	.093	3.90	3.009	.246	8.17

[1] % C.V. = Standard deviation/mean × 100

TABLE VIII.- FLUORESCENCE RATIOS FROM TECHNIQUE OF REFERENCE 8

Species	$\dfrac{F^*(539)}{F^*(454)}$	$\dfrac{F^*(598)}{F^*(539)}$	$\dfrac{F^*(617)}{F^*(539)}$
Va-9 (blue-green)	2.93	4.92	8.06
Va-12 (golden-brown)	.44	.74	1.12
Va-13 (dinoflagellate)	.67	.52	.65
Va-72 (diatom)	.71	.47	.68
Va-74 (green)	.29	2.50	3.08
Va-70 (red)	8.98	.27	.34

23

Refrigerator

High voltage
supply

Charging
network

H$_2$O and dye pumps
bubble filters
heat exchanger

Trigger
generator

Capacitor

Laser
head

PMT
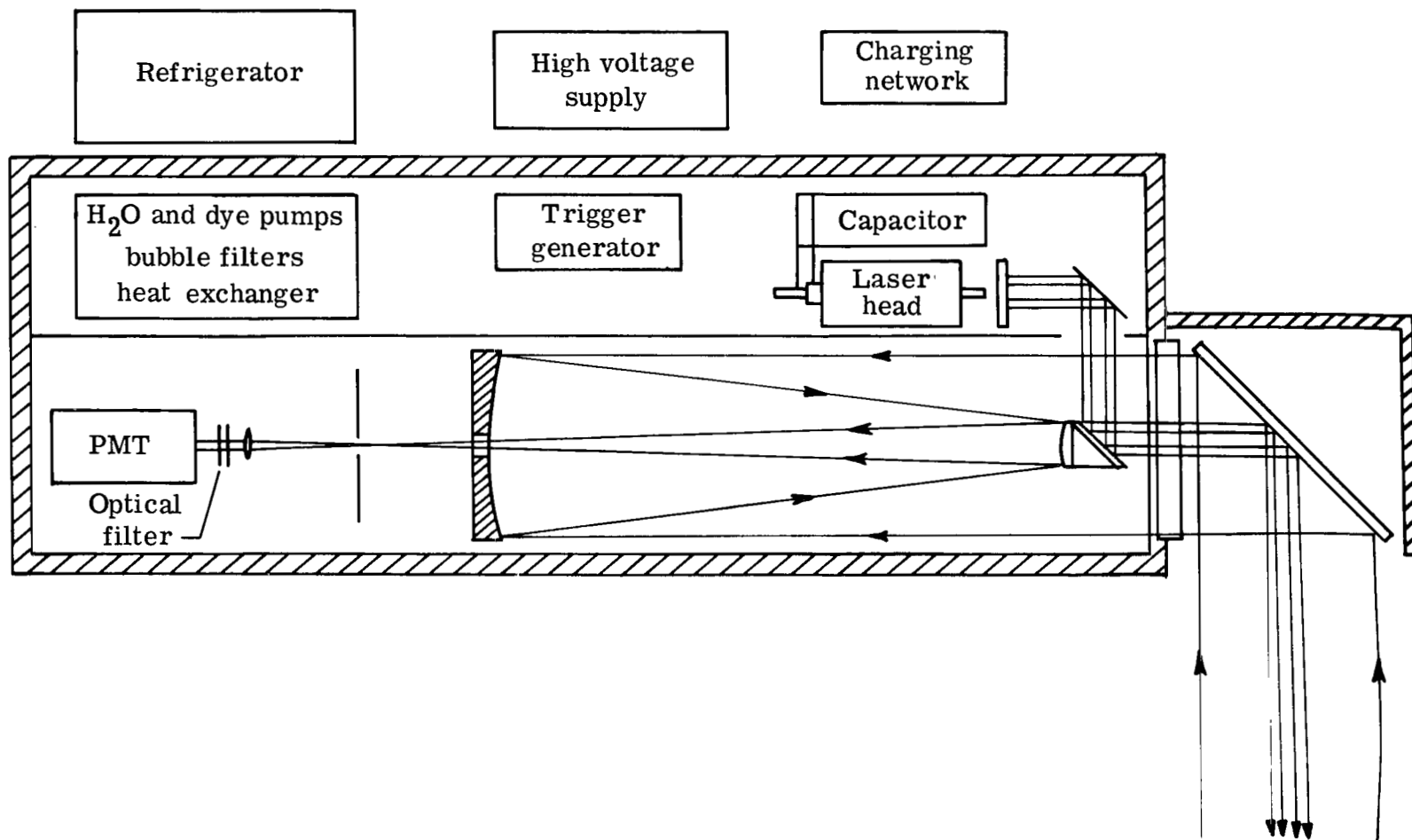
Optical
filter

Figure 1.- Schematic of fluorosensor.
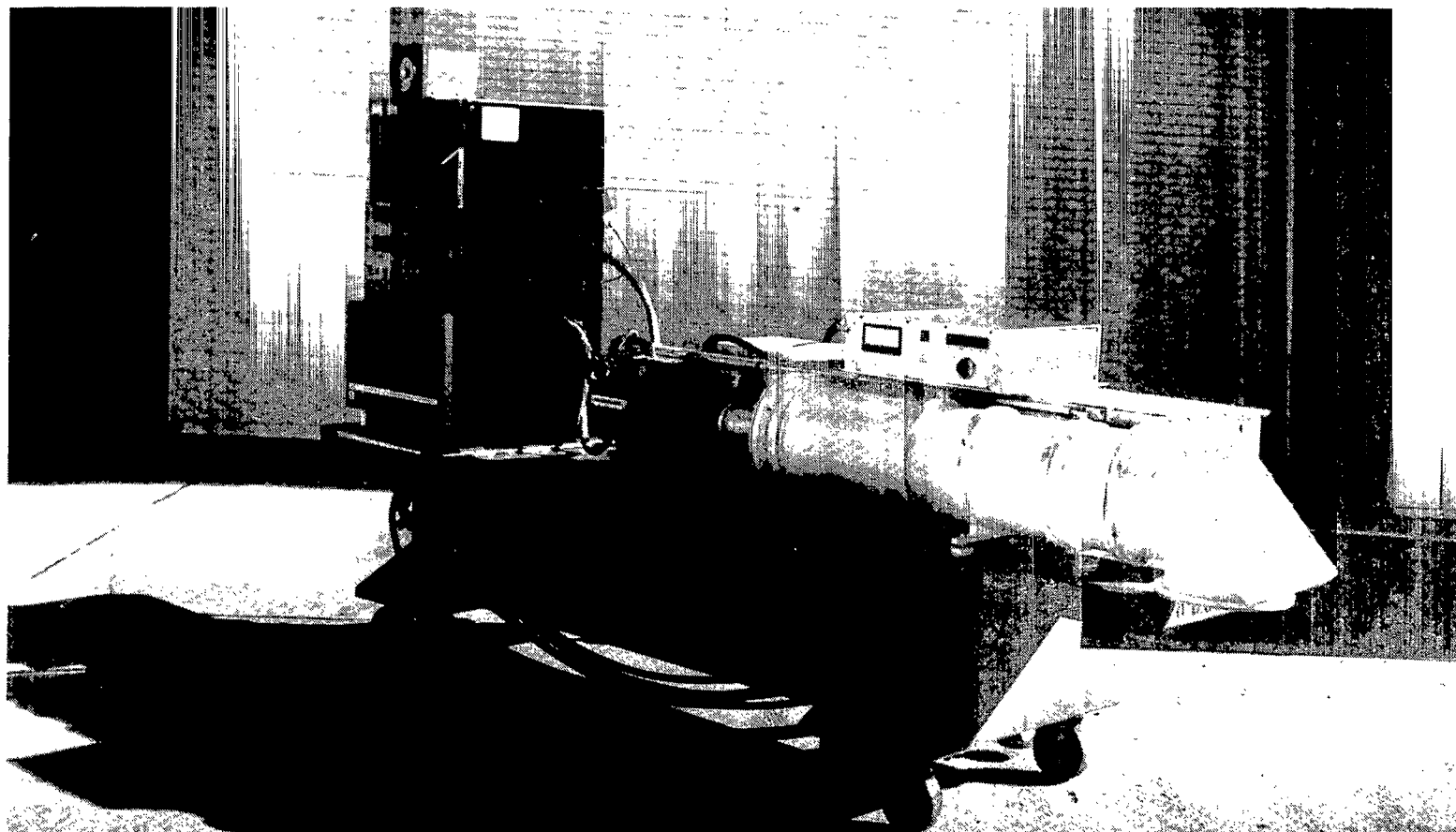
L-79-1953

(a) Laser and telescope.

Figure 2.- Photographs of fluorosensor system.
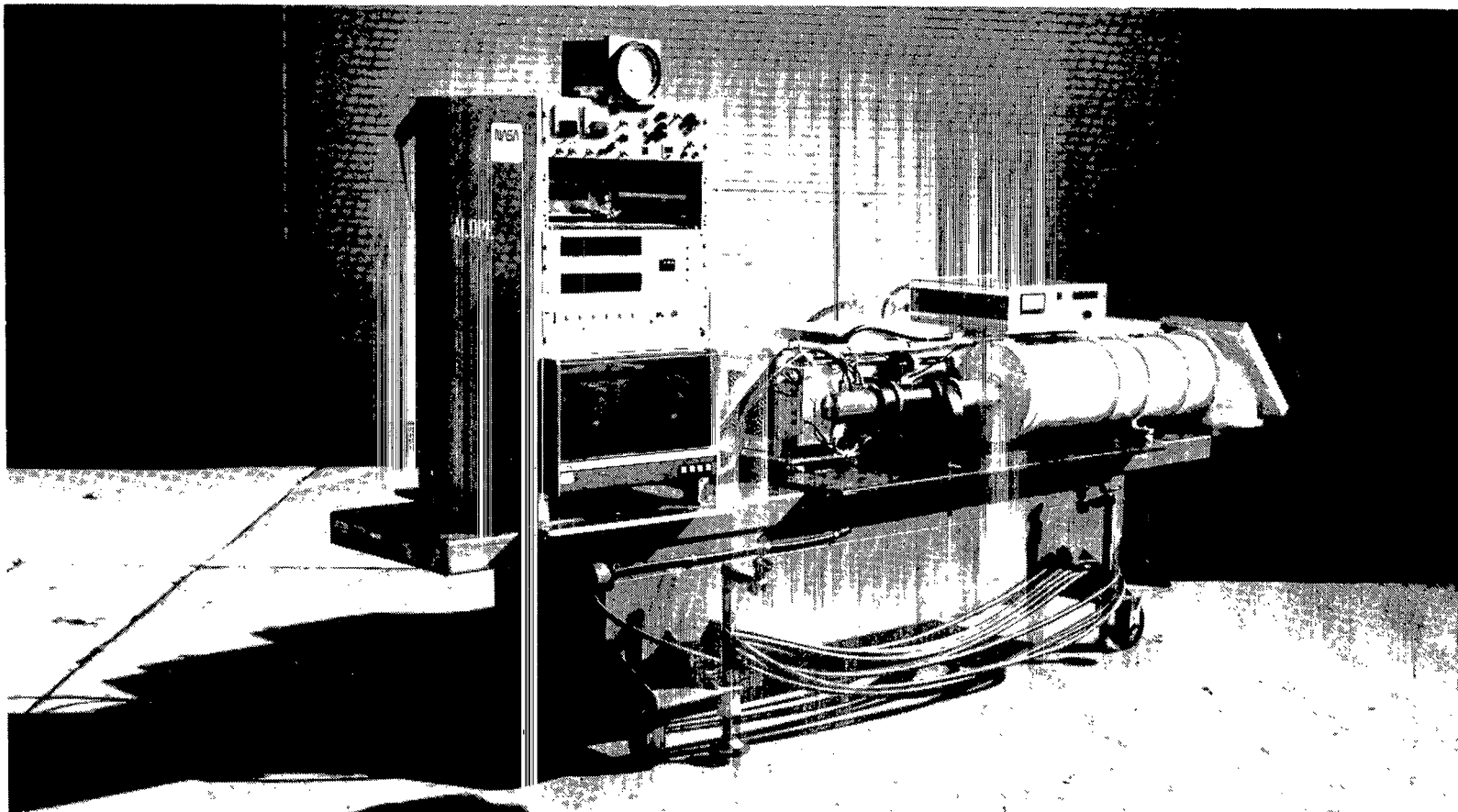
L-79-1955

(b) Control panel.

Figure 2.- Concluded.
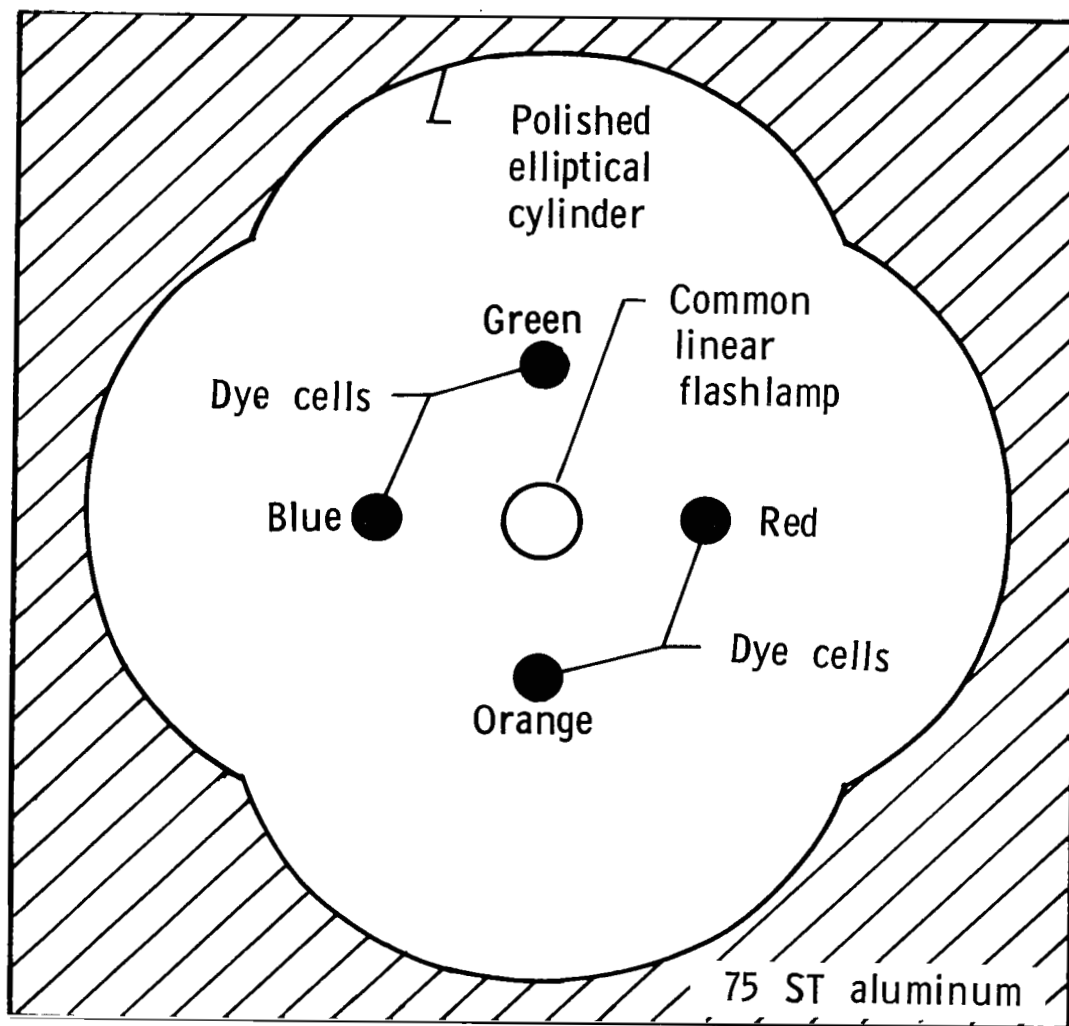
Figure 3.- Cross-sectional view of multielliptical cavity showing location of dye cells and linear flash lamp.
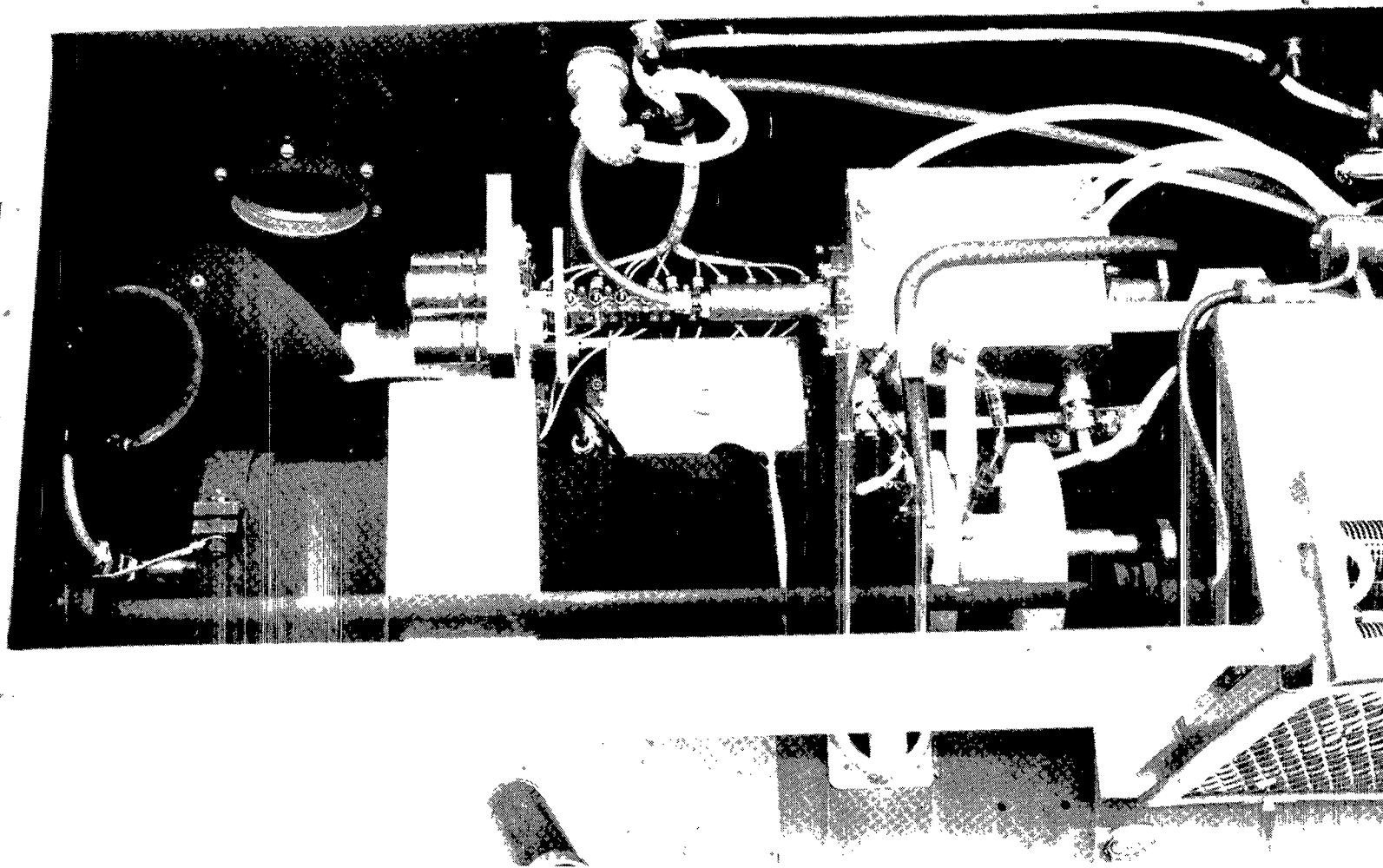
Figure 4.- Photograph of laser mounted in system.

L-73-3192
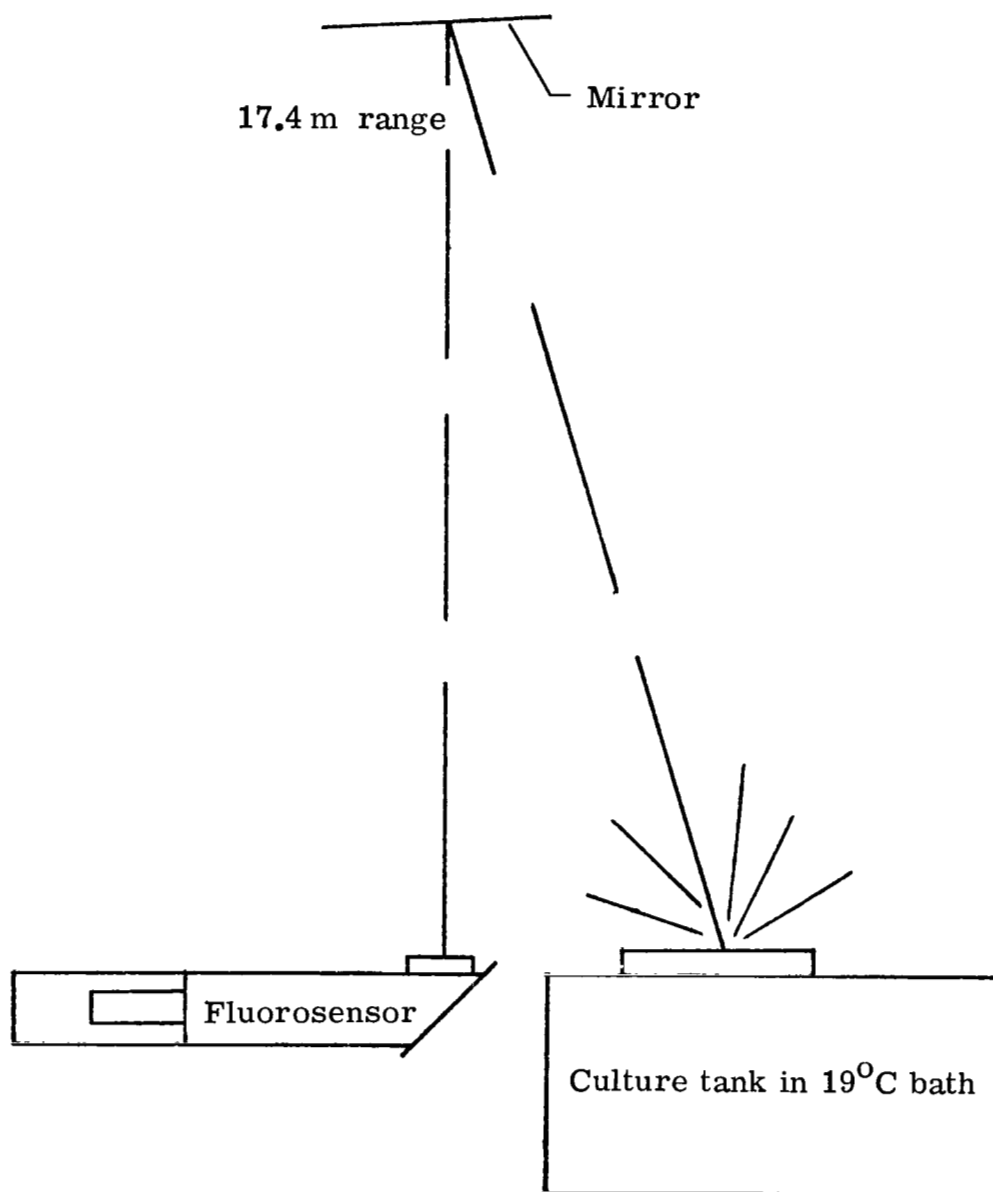
Figure 5.- Schematic of fluorosensor in laboratory.
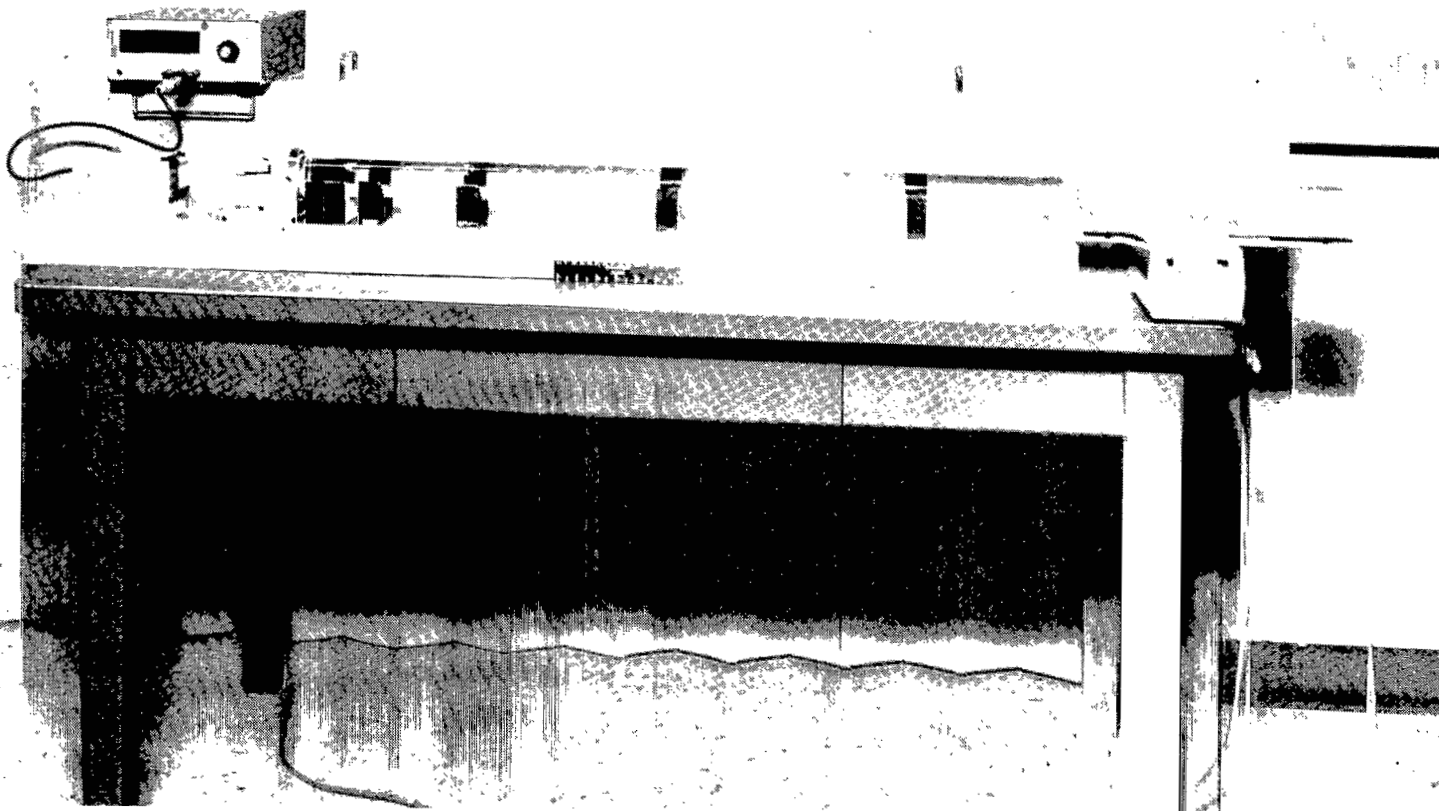
L-76-1594

Figure 6.- Photograph of helium-neon laser and tube for determination
of effective attenuation coefficient.
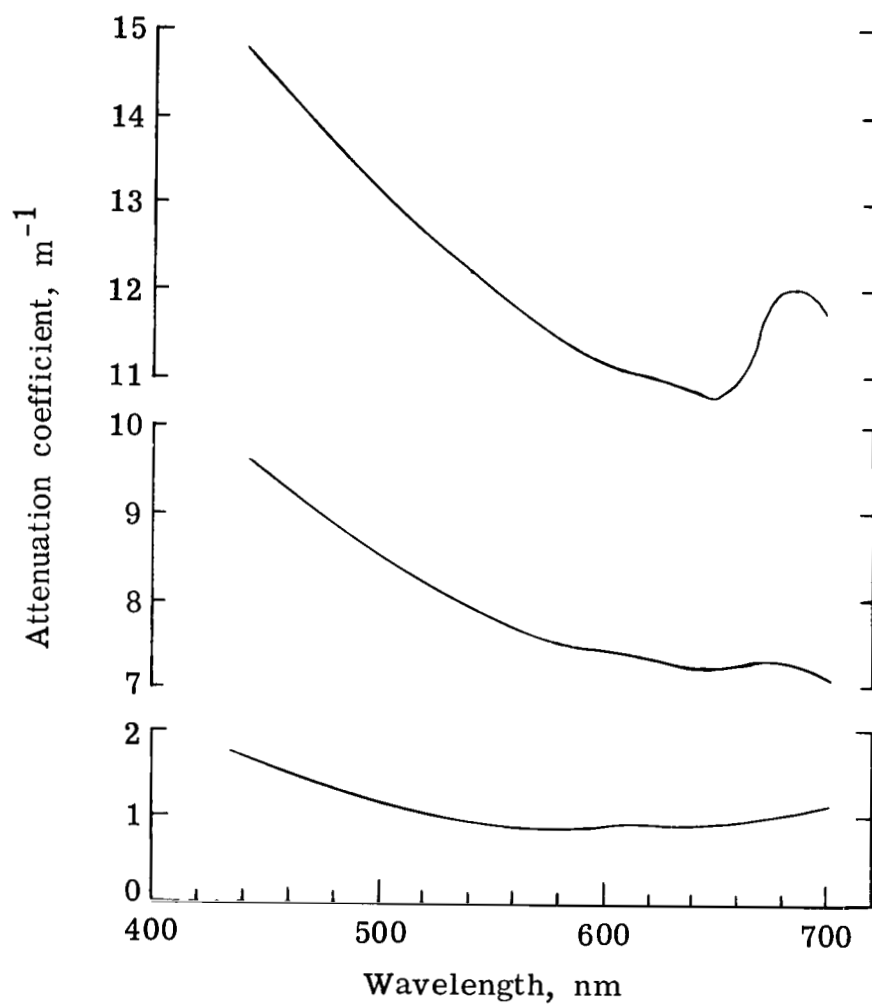
Figure 7.- Representative composite curves of effective light attenuation coefficient, α, obtained with a transmission spectrophotometer.

Energy received (at wavelength λ_f) when excited at wavelength λ_i, J

$$P_r(\lambda_f, \lambda_i) = \left[\frac{\xi A}{4\pi R^2 m^2} \left(\frac{\Delta\lambda_D}{\Delta\lambda_f} \right) \left(\frac{\theta_r}{\theta_L} \right)^2 \right] \left[\frac{P_0(\lambda_i)}{(\alpha_f + \alpha_i)} \right] \left[\sigma(\lambda_i) n \right] \left[1 - \frac{(mR)^2}{(mR+d)^2} e^{-(\alpha_f + \alpha_i)d} \right]$$

Measurable constants related to sensor geometry, sensitivity, etc.	Parameters dependent on excitation wavelength λ_i	Fluorescence term	Finite depth term
ξ = total optical efficiency (0.226)	$P_0(\lambda_i)$ = laser energy output at wavelength λ_i, J	$\sigma(\lambda_i)$ = fluorescence cross section at λ_i, m^2/molecule	d = depth of culture (water column depth), m
A = effective area of receiving telescope primary mirror (0.0380 m^2)	α_f = attenuation coefficient of water at 685 nm, m^{-1}	n = molecular density of chlorophyll <u>a</u>, molecules/m^3	
$\Delta\lambda_D$ = spectral width of detector (see note 1), nm			
$\Delta\lambda_f$ = spectral width of fluorescence (see note 1), nm	α_i = attenuation coefficient of water at wavelength λ_i, m^{-1}		
θ_r = receiver field of view (see note 2), sr			
θ_L = beam divergence of laser (see note 2), sr			
R = distance from laser to water (17.4 m)			
m = index of refraction (1.333)			

Note 1. The value of $\Delta\lambda_D / \Delta\lambda_f = 0.54$

Note 2. The value of $\theta_r / \theta_L = 1$

Figure 8.- Fluorosensor equation.
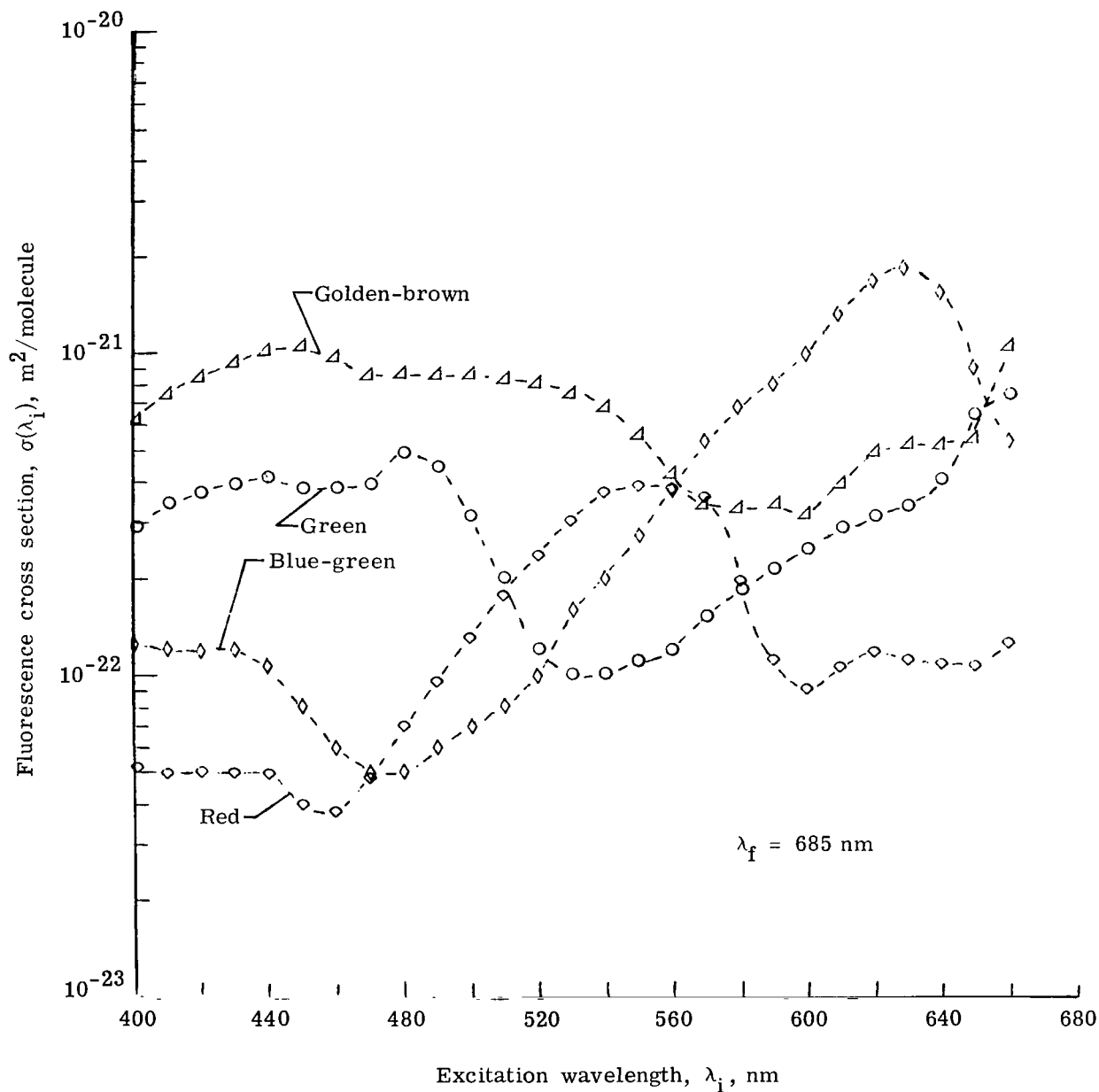
Figure 9.- Average fluorescence cross section values representative of four color groups as determined by a fluorescence spectrophotometer system using the method of reference 8.
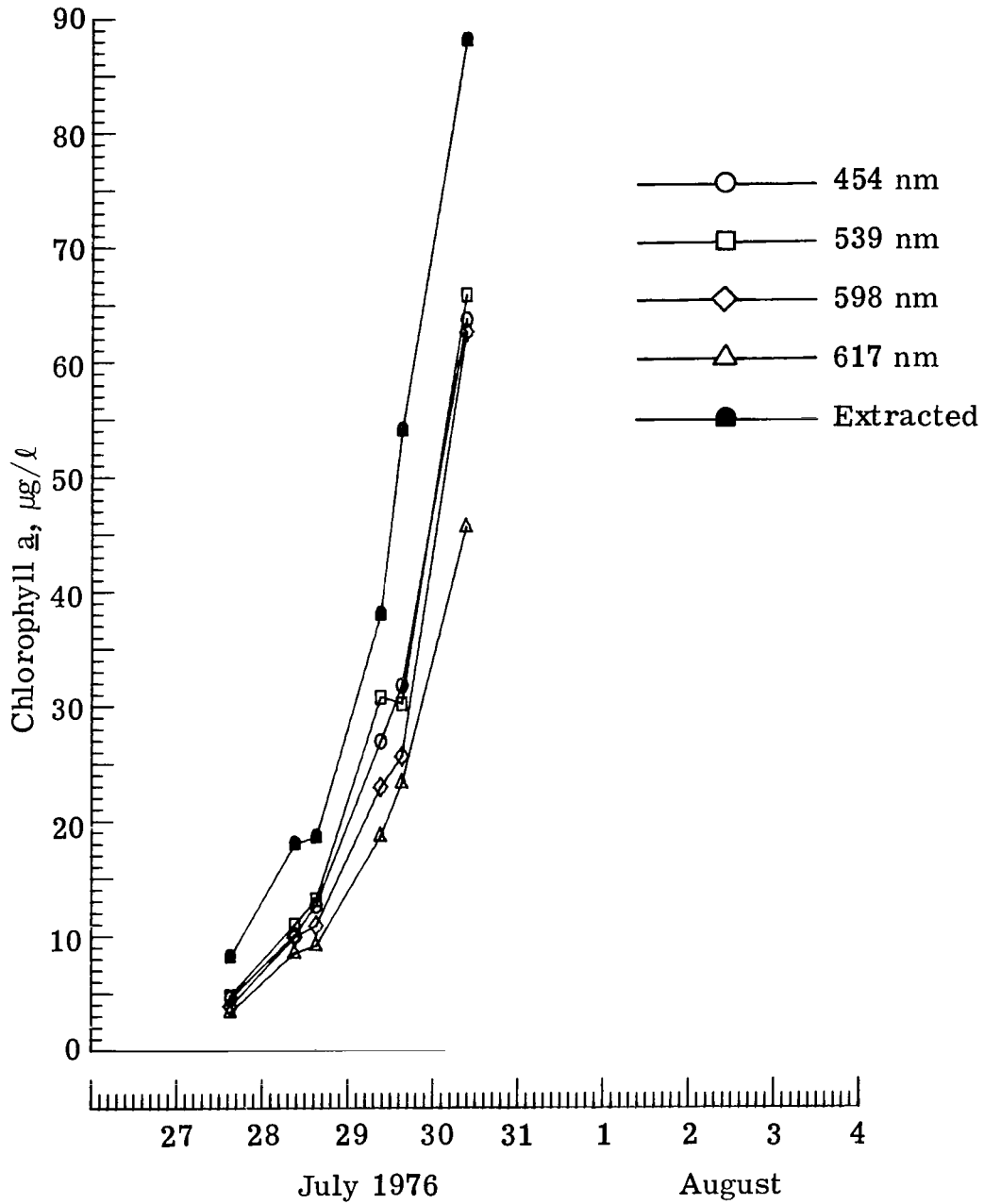
(a) Va-12 species, July 26 to 30, 1976.

Figure 10.- Variation of remote estimates and extracted chlorophyll a with time for golden-brown algae, Va-12.
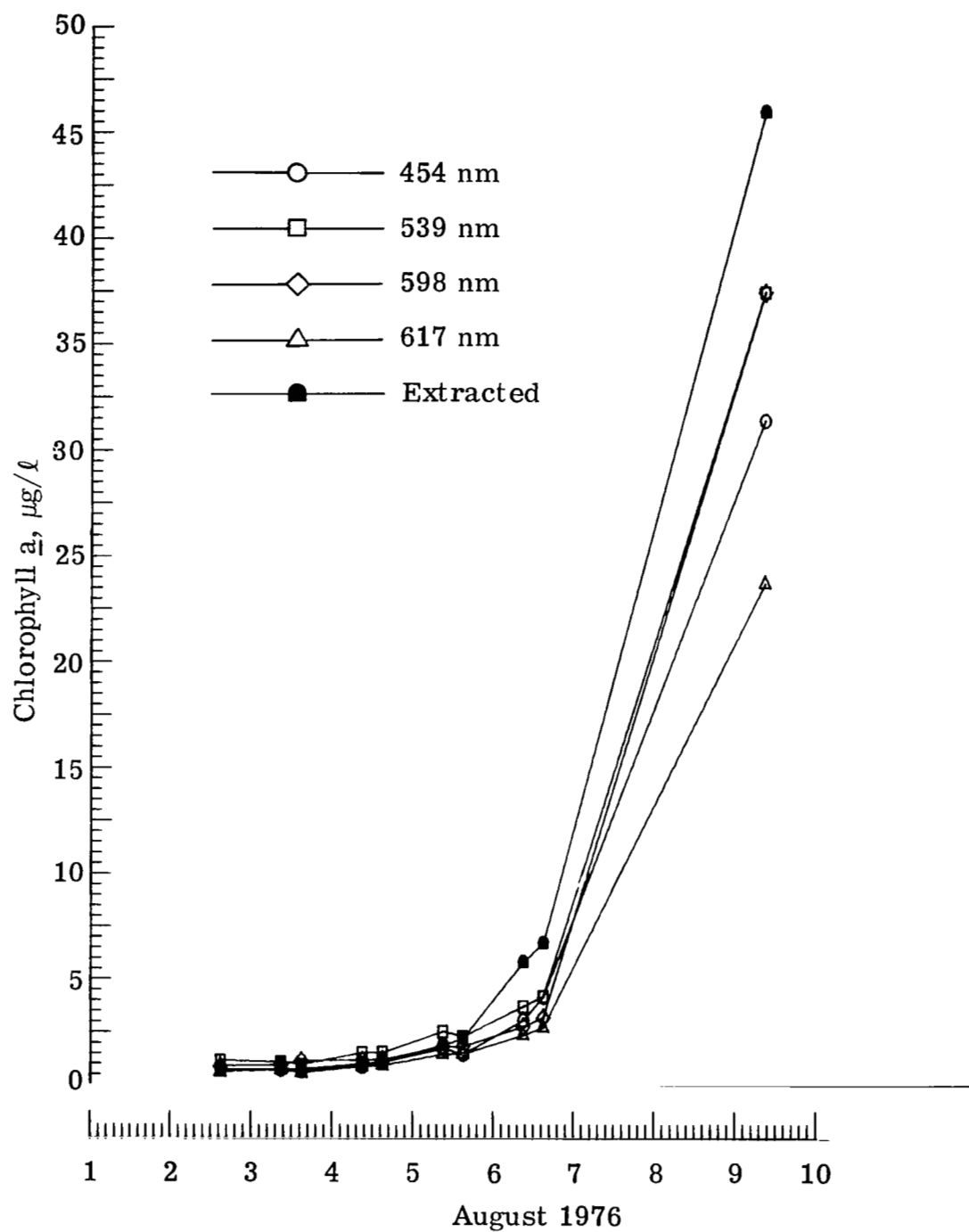
(b) Va-12 species, August 2 to 9, 1976.

Figure 10.- Continued.
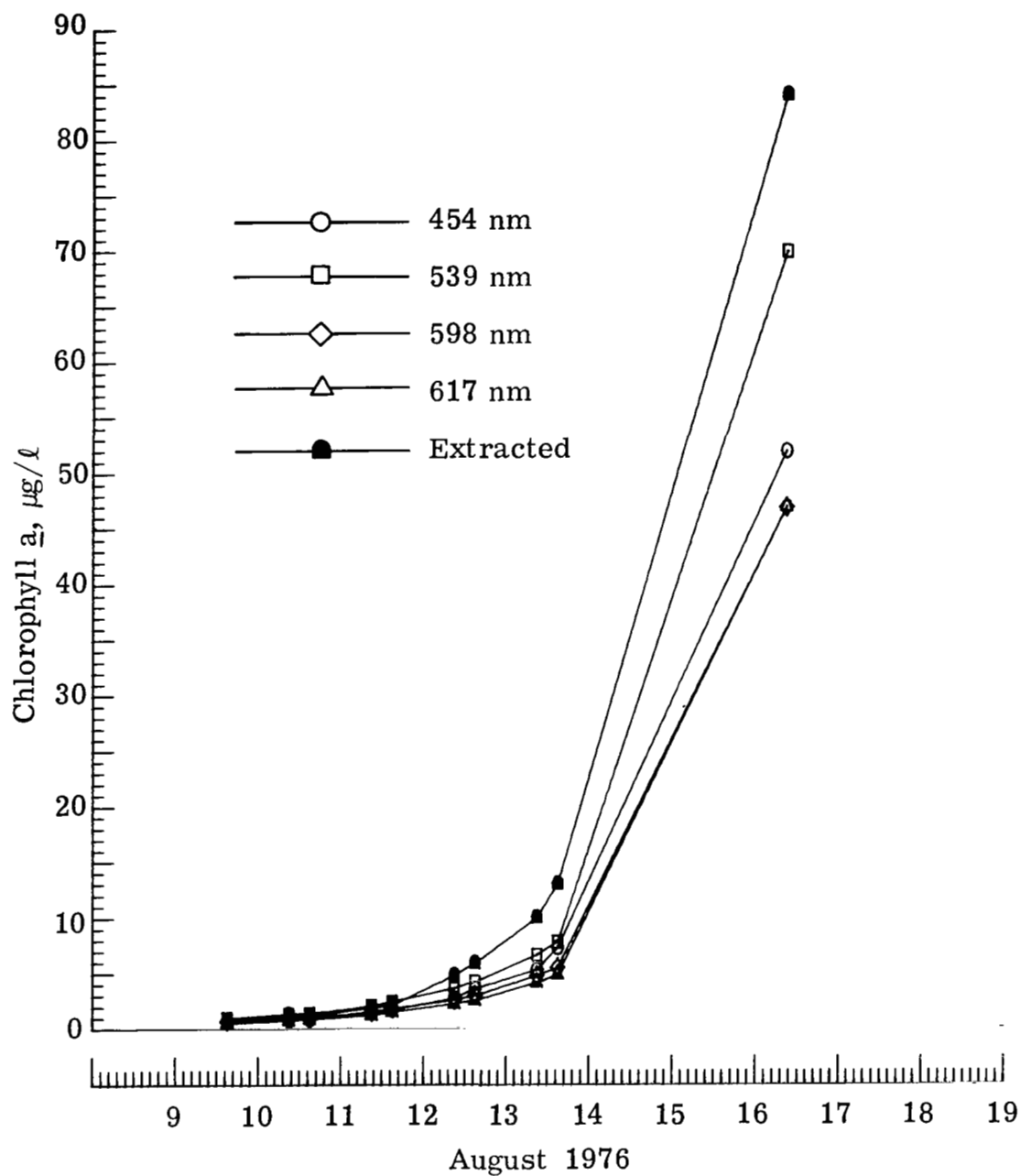
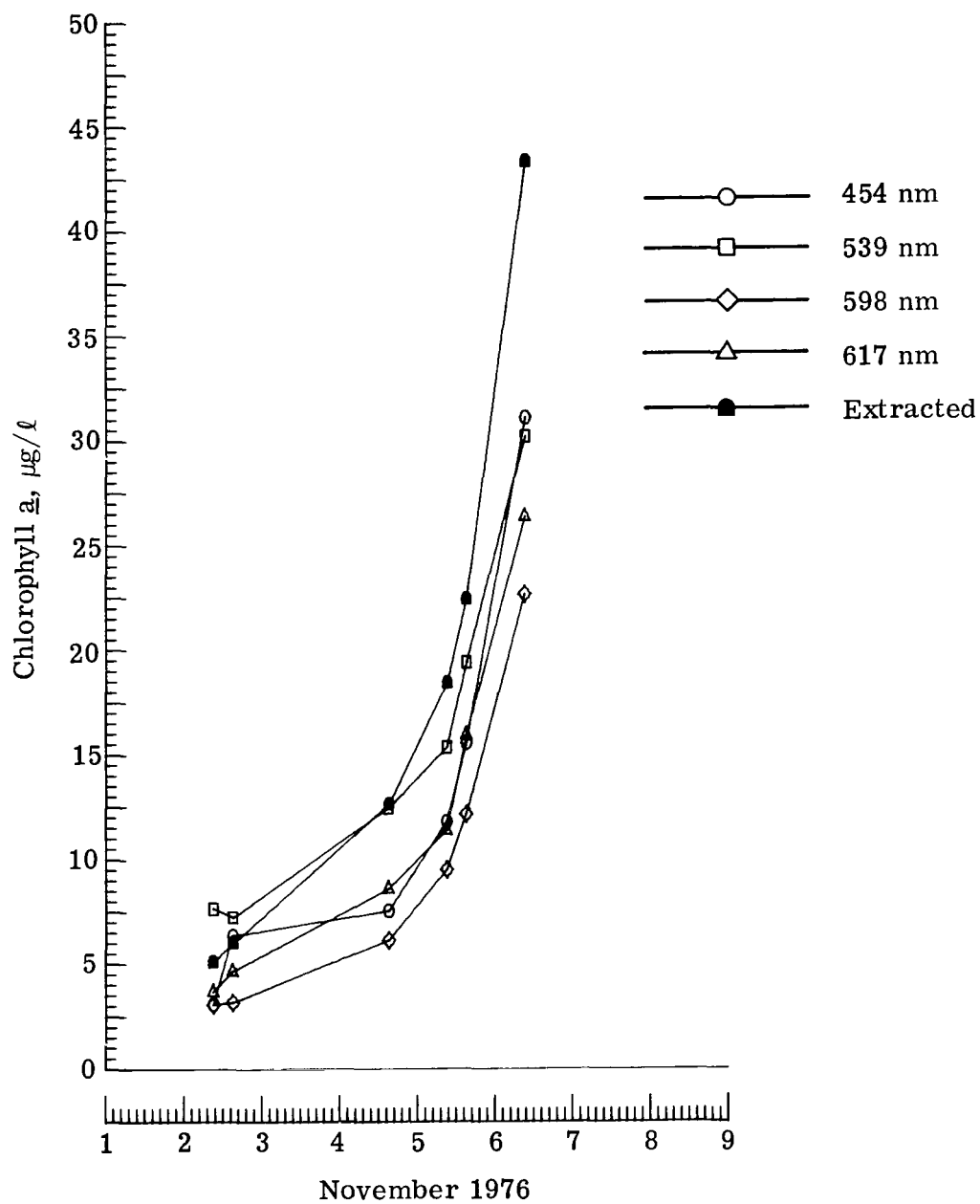(c) Va-12 species, August 9 to 16, 1976.

Figure 10.- Concluded.
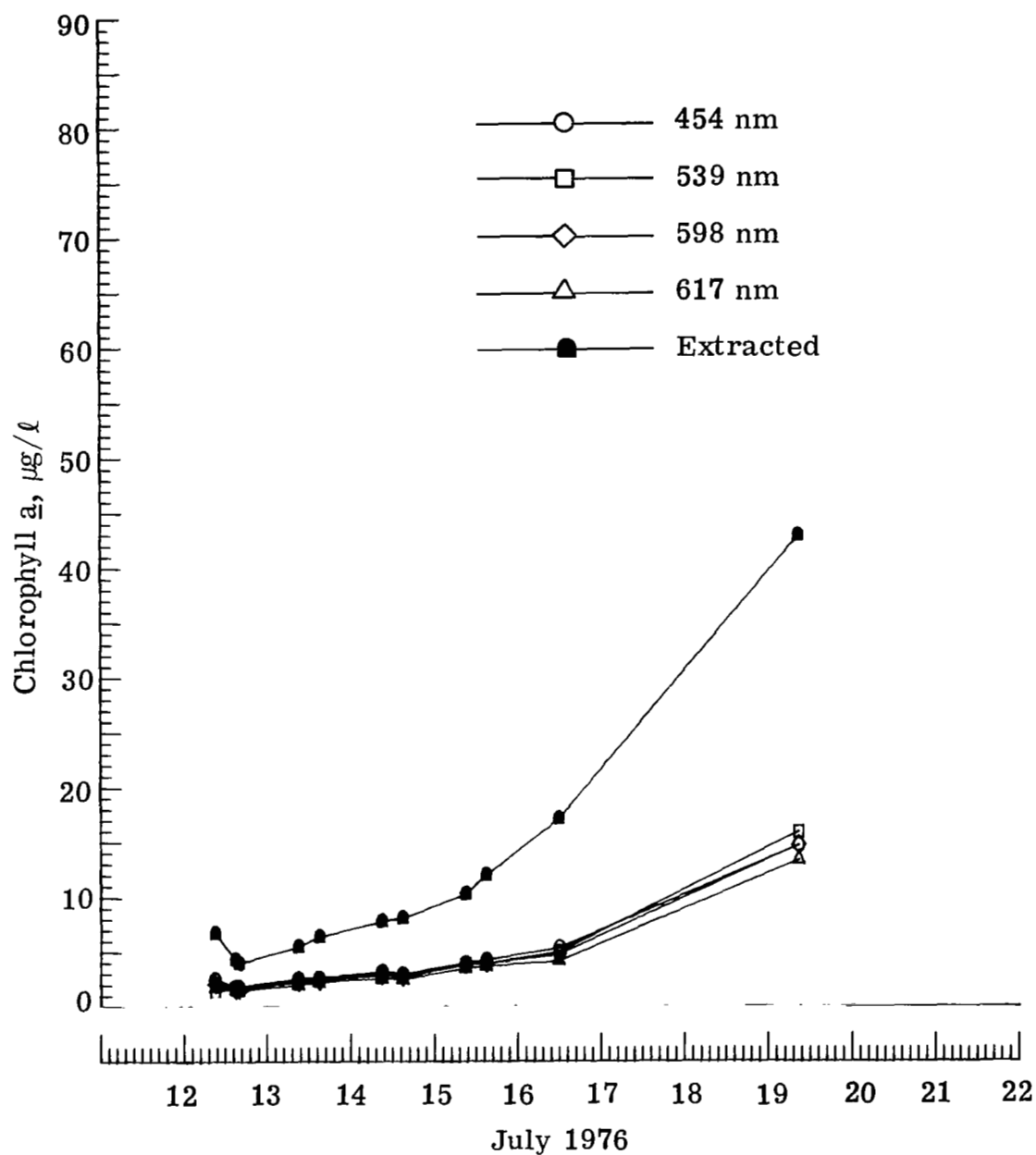
(a) Va-13 species, July 9 to 19, 1976.

Figure 11.- Variation of remote estimates and extracted chlorophyll a with time for dinoflagellate, Va-13.
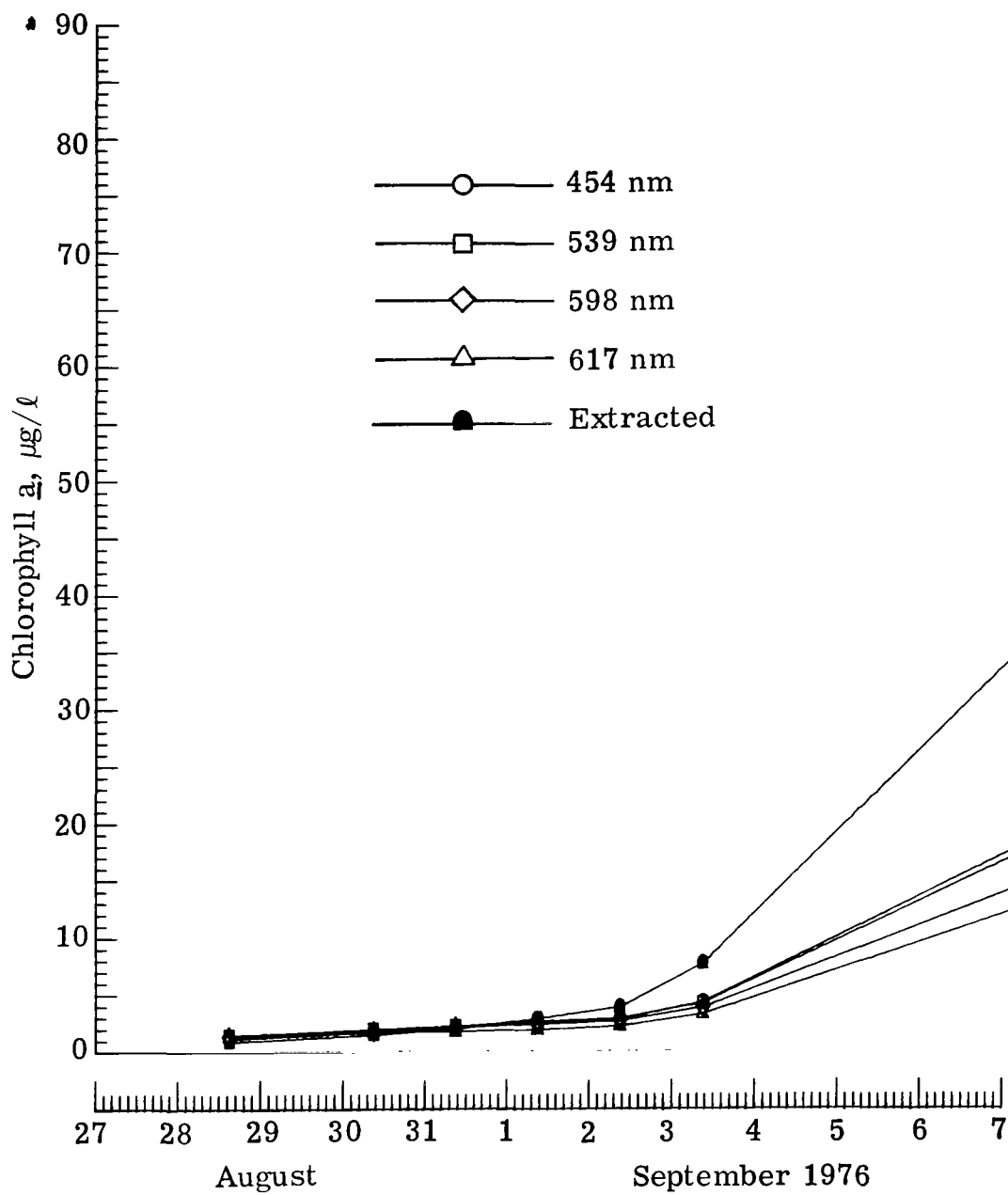
(b) Va-13 species, August 27 to September 7, 1976.

Figure 11.- Continued.
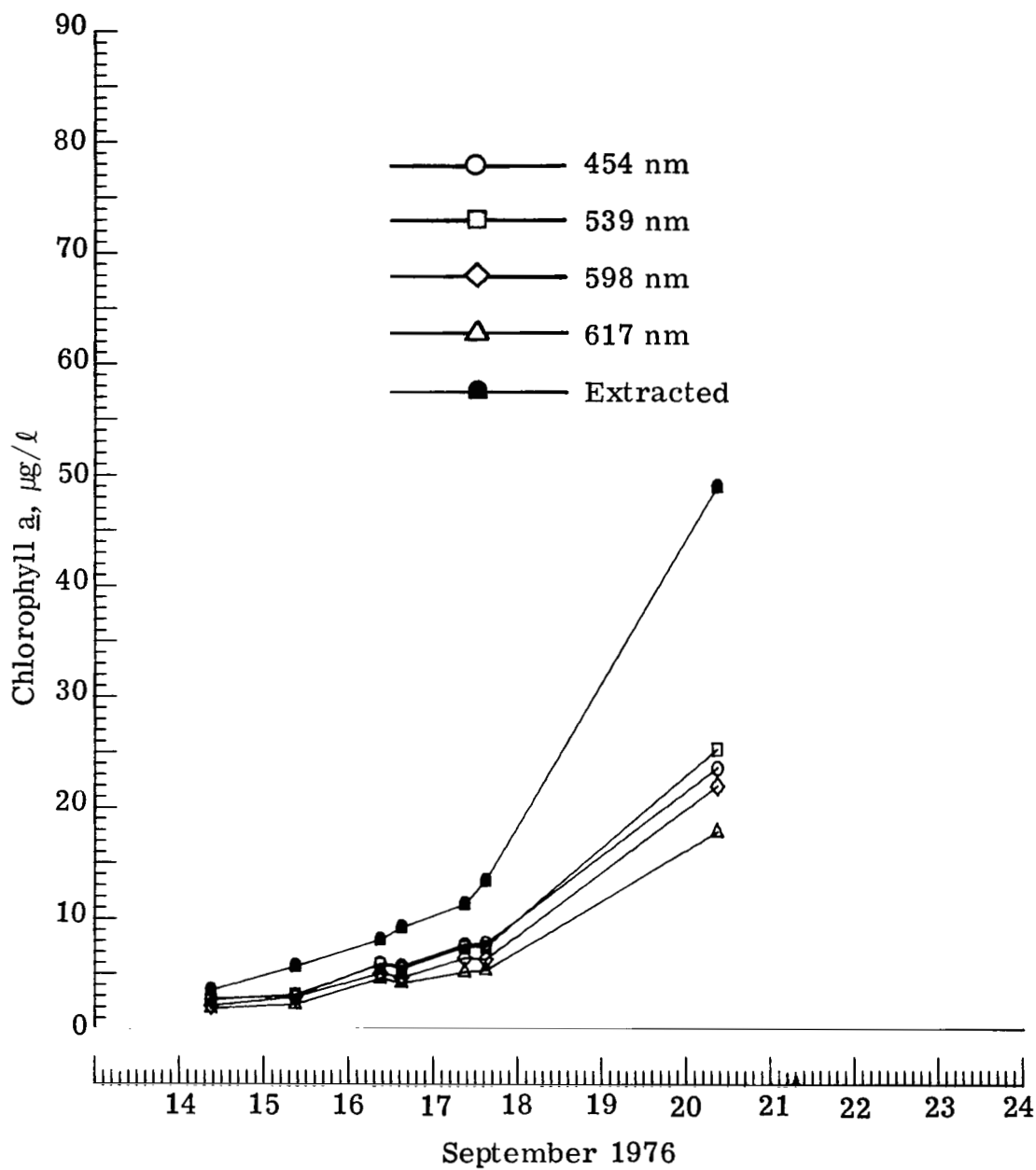
(c) Va-13 species, September 13 to 20, 1976.

Figure 11.- Concluded.
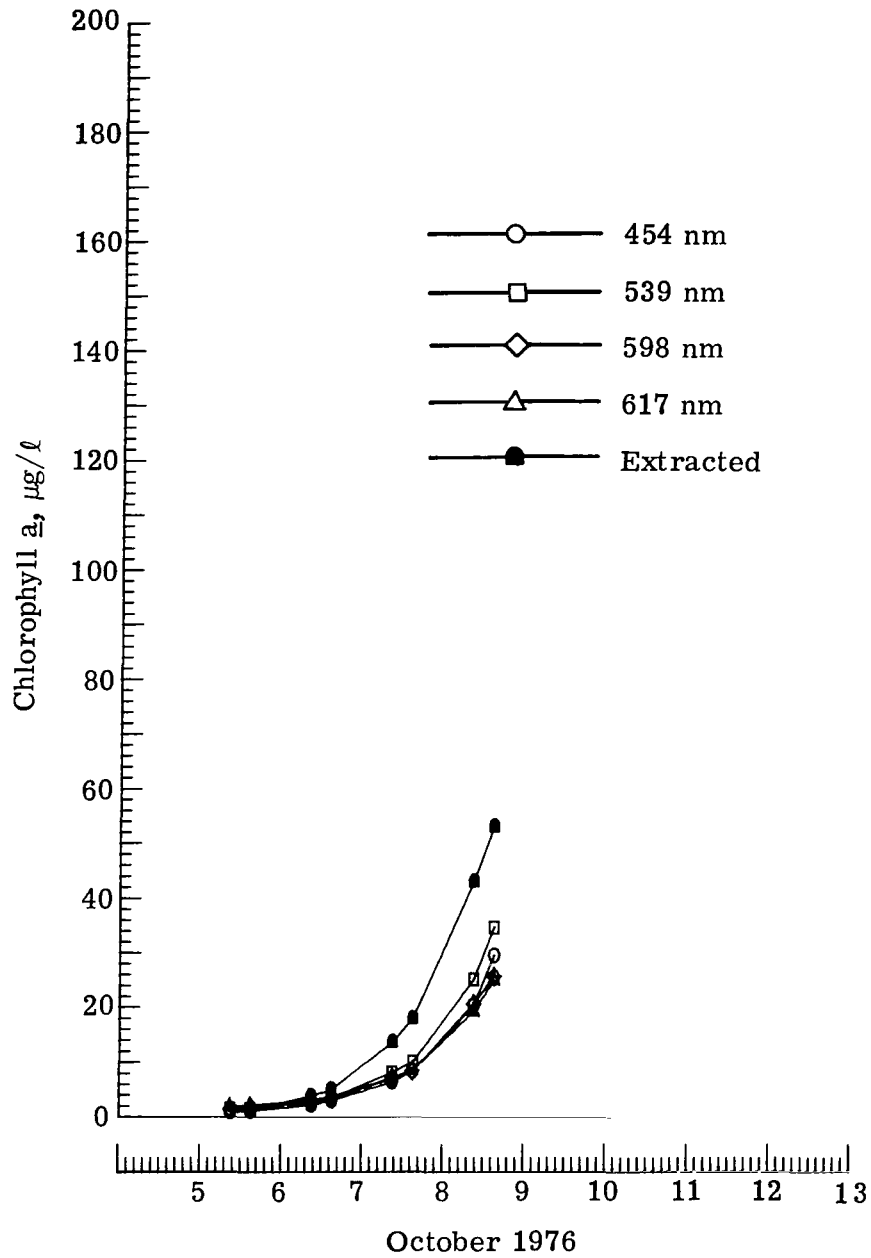
(a) Va-72 species, October 5 to 8, 1976.

Figure 12.- Variation of remote estimates and extracted chlorophyll a with time for diatom, Va-72.
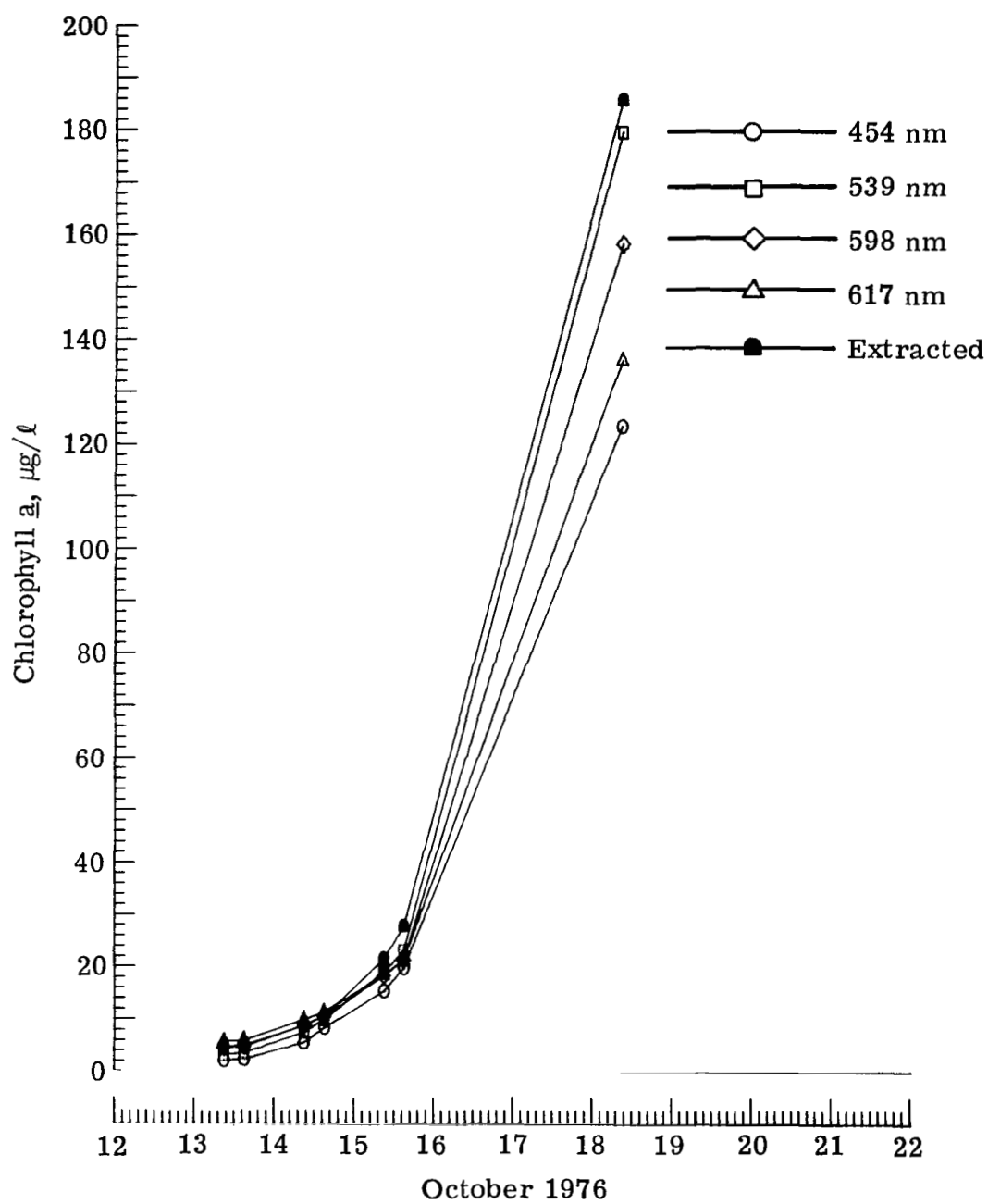
(b) Va-72 species, October 12 to 19, 1976.

Figure 12.- Continued.
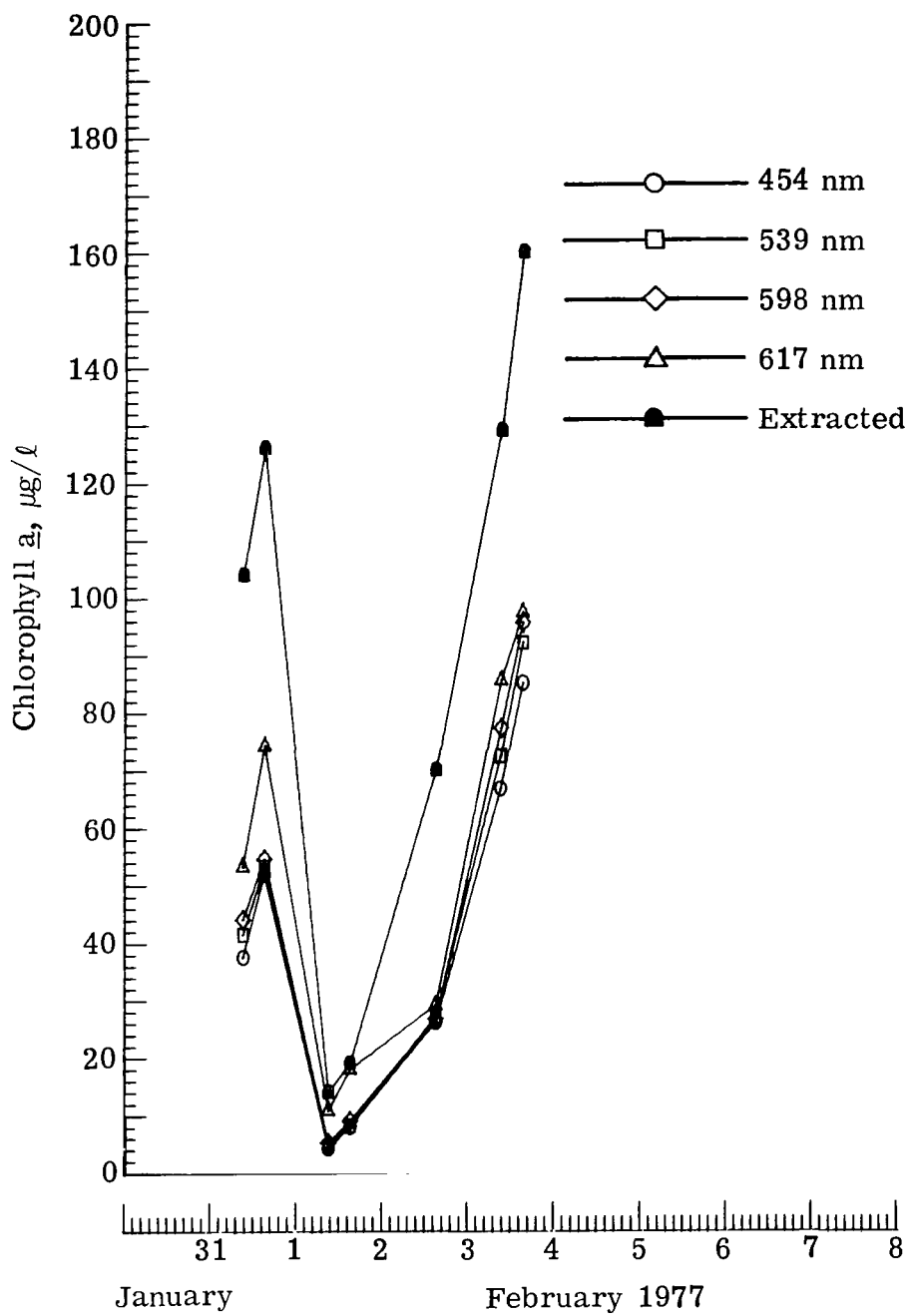
(c) Va-72 species, January 28 to February 3, 1977.

Figure 12.- Continued.
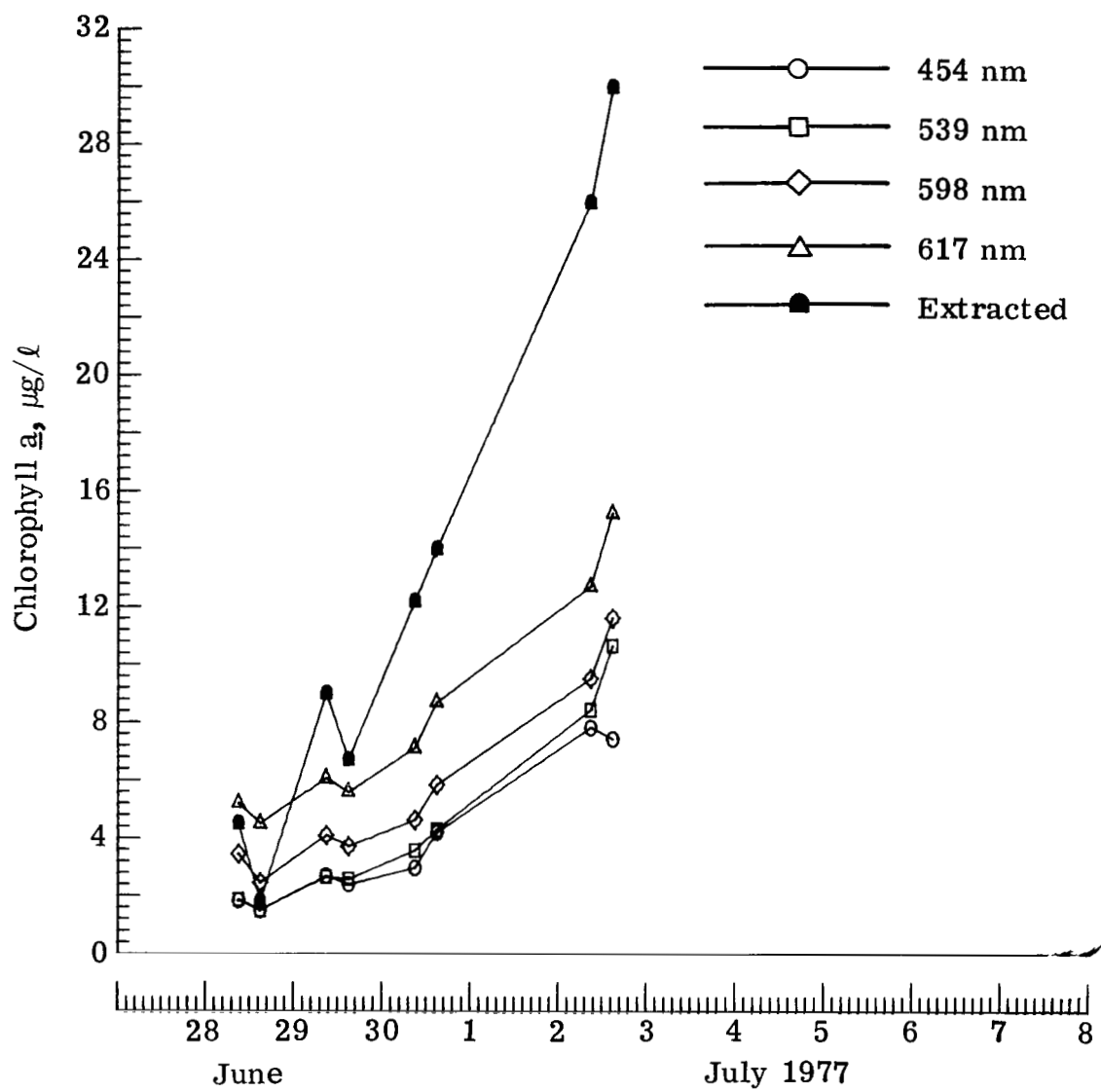
(d) Va-72 species, June 28 to July 2, 1977.

Figure 12.- Concluded.
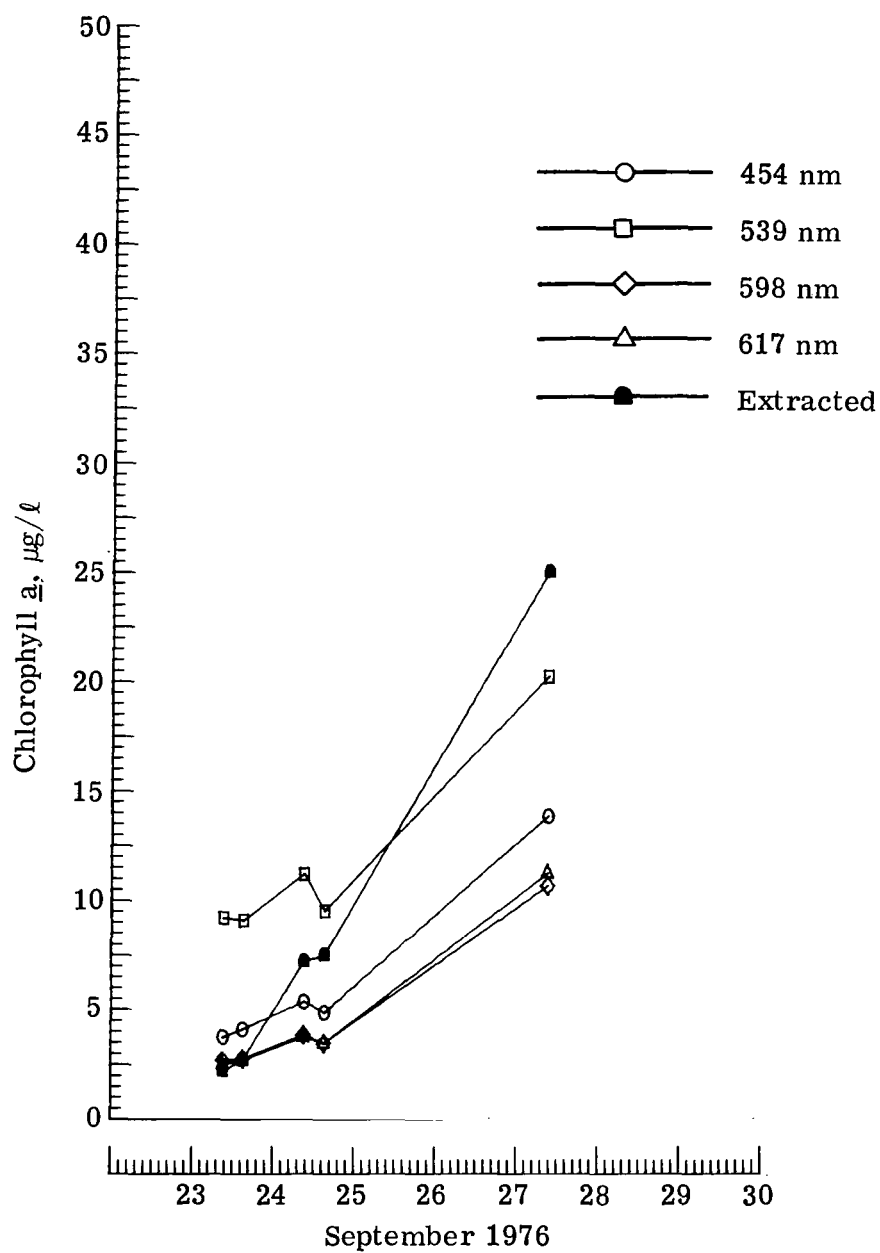
(a) Va-74 species, September 20 to 27, 1976.

Figure 13.- Variation of remote estimates and extracted chlorophyll $\underline{a}$ with time for green algae, Va-74.
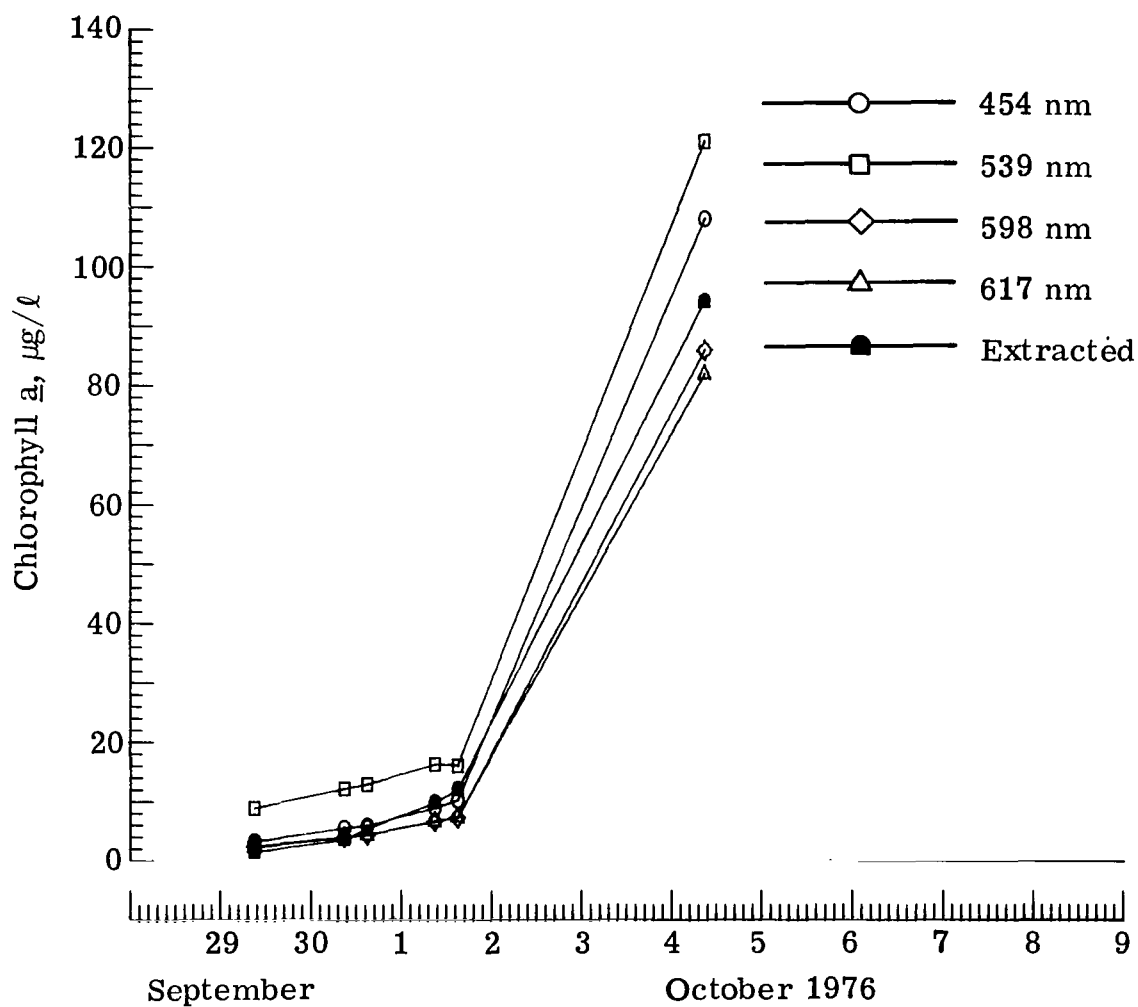
(b) Va-74 species, September 27 to October 4, 1976.

Figure 13.- Continued.

(c) Va-74 species, November 1 to 6, 1976.

Figure 13.- Continued.
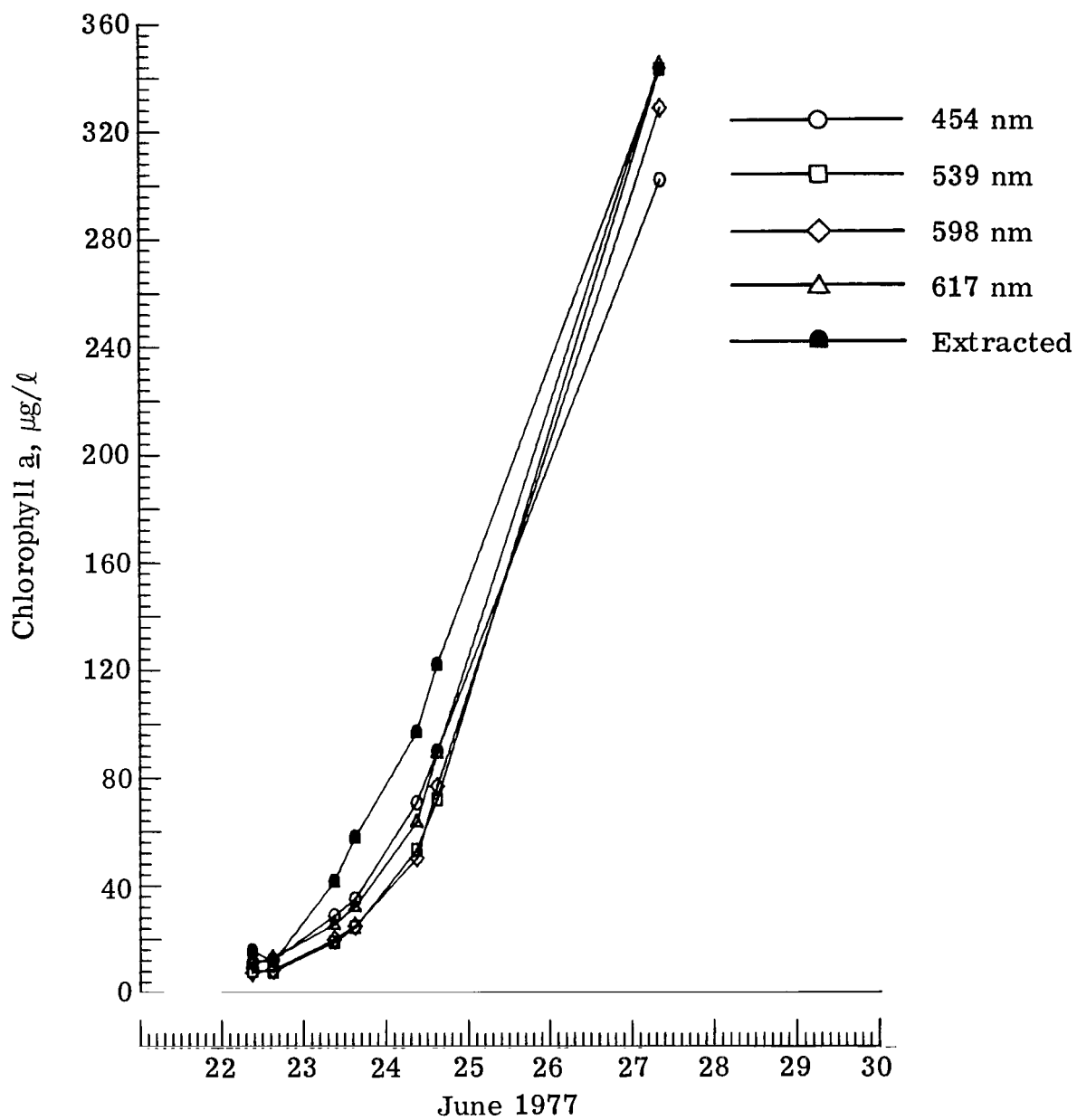
(d) Va-74 species, June 22 to 27, 1977.

Figure 13.- Concluded.
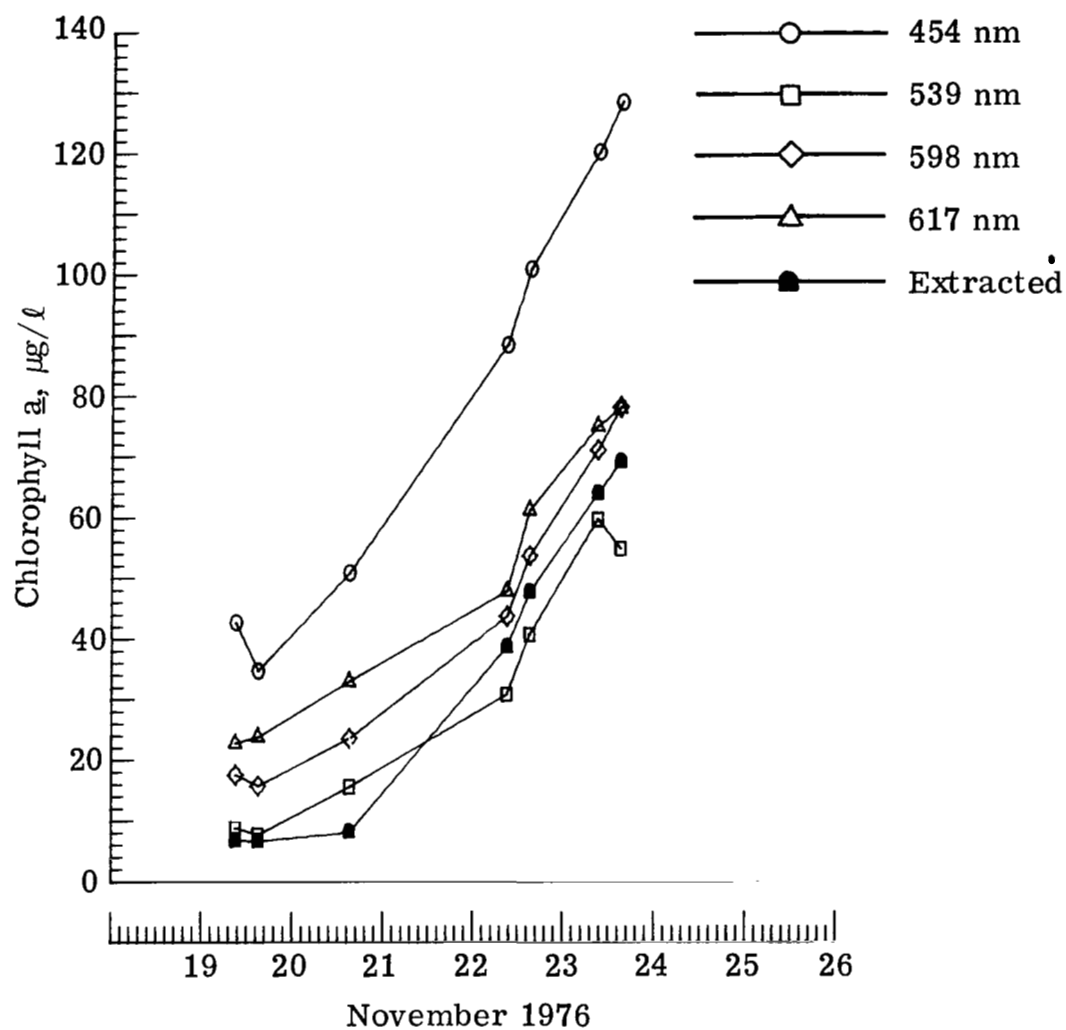
(a) Va-70 species, November 17 to 23, 1976.

Figure 14.- Variation of remote estimates and extracted chlorophyll a with time for red algae, Va-70.
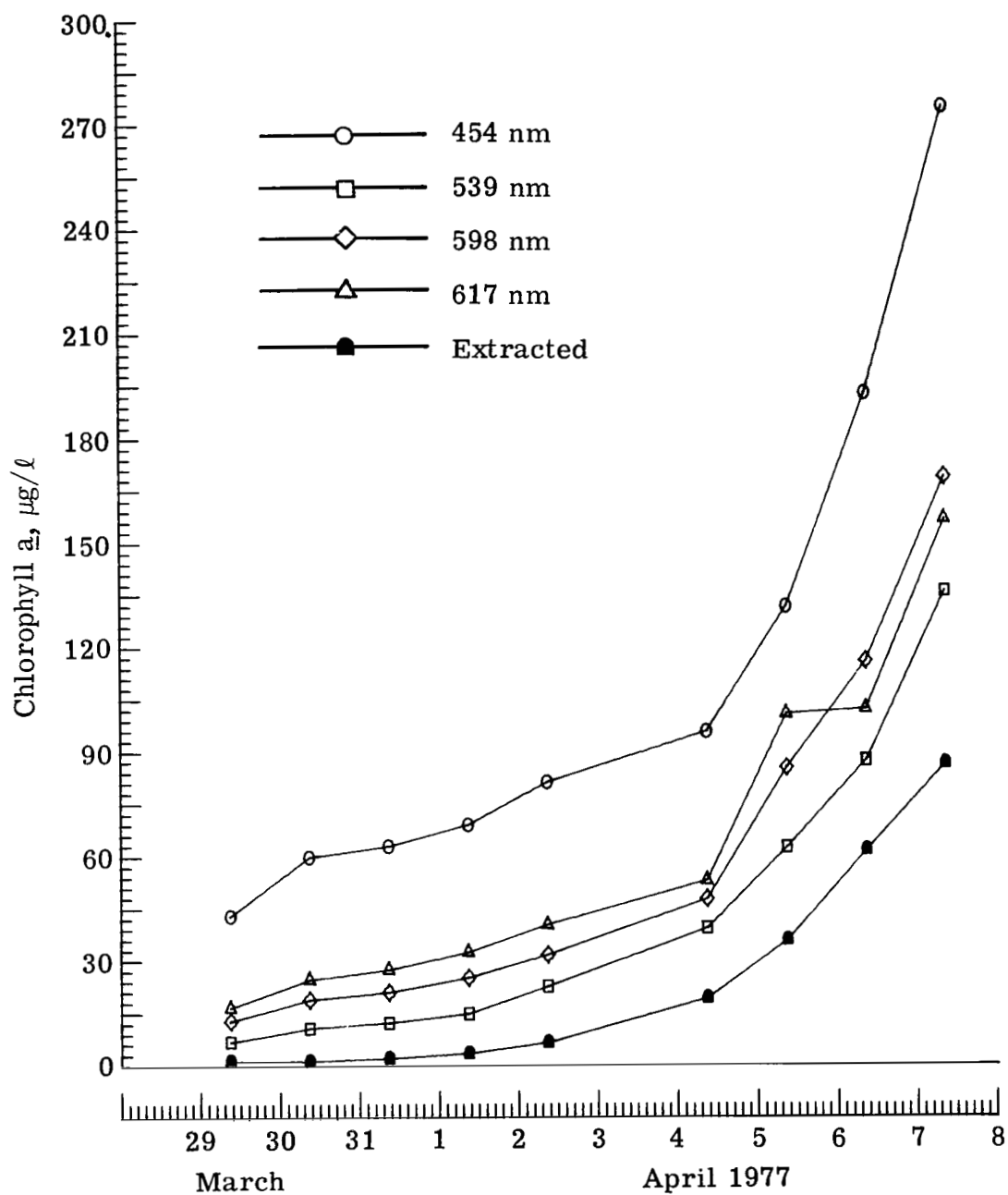
(b) Va-70 species, March 28 to April 7, 1977.

Figure 14.- Concluded.
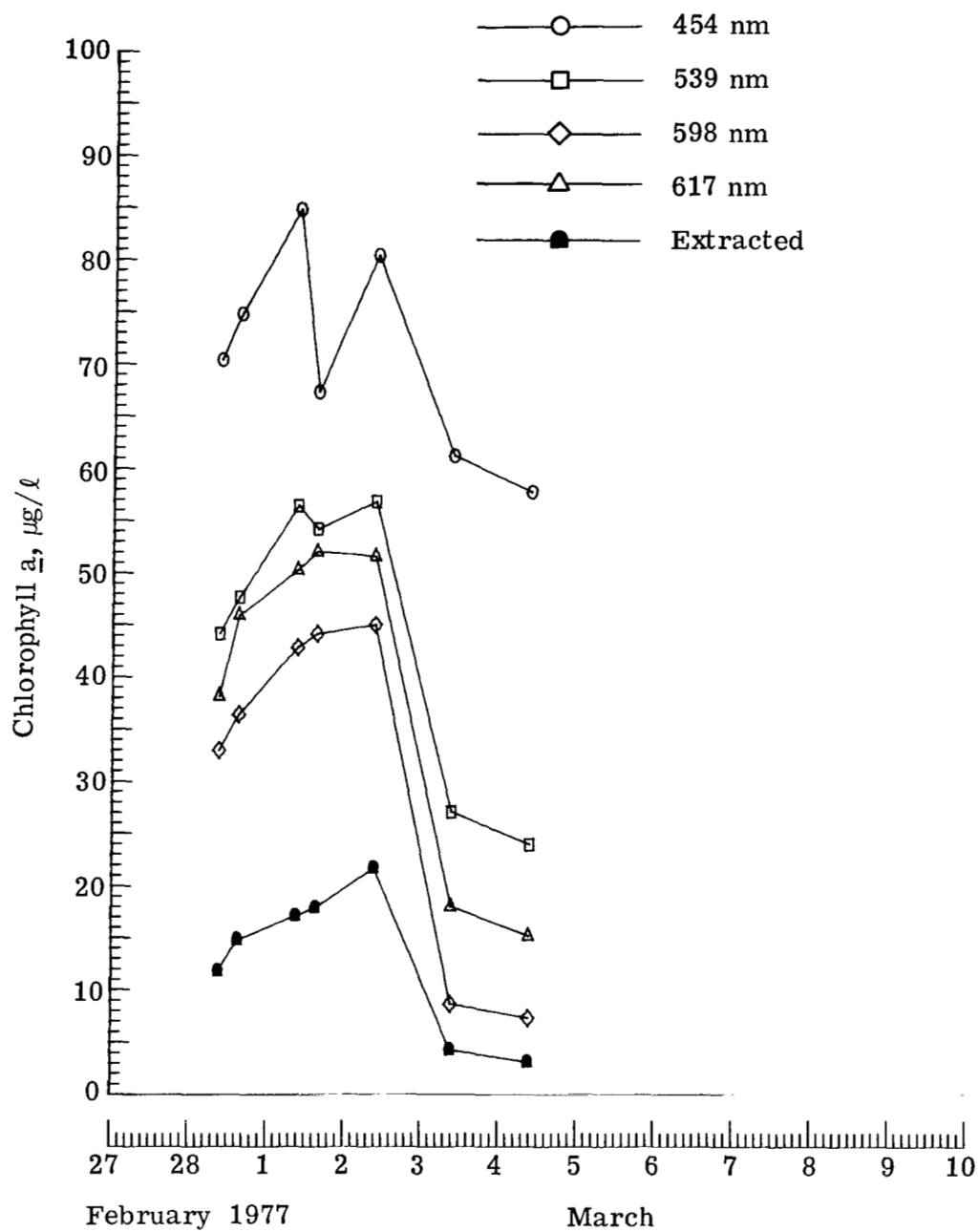
(a) Va-9 species, February 25 to March 4, 1977.

Figure 15.- Variation of remote estimates and extracted chlorophyll a with time for blue-green algae, Va-9.
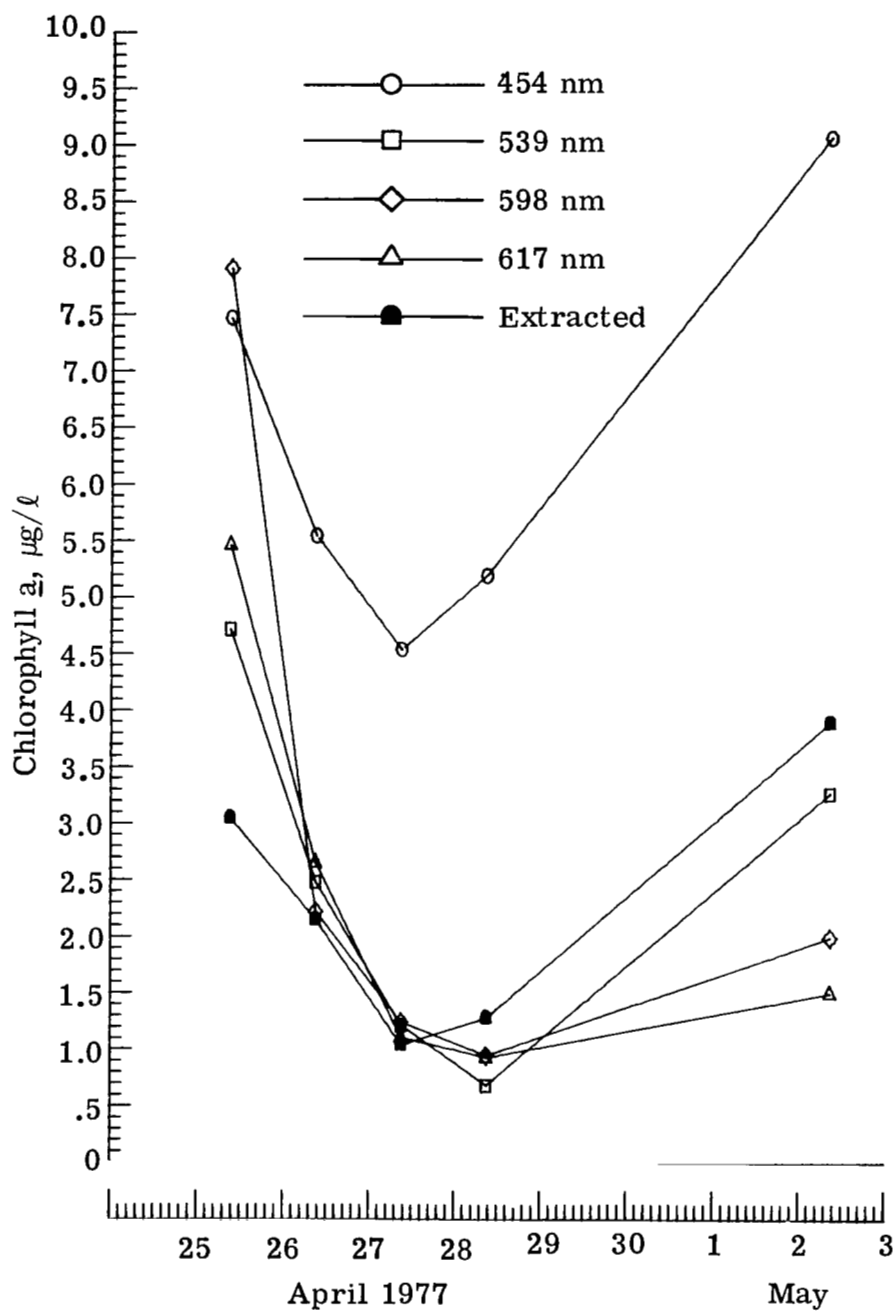
(b) Va-9 species, April 25 to May 3, 1977.

Figure 15.- Concluded.

		r
——○——	454 nm $y = 5.47 \times 10^{-22}x - 15.71 \times 10^{-22}$	.9922
——□——	539 nm $y = 33.47 \times 10^{-23}x - 6.83 \times 10^{-22}$	.9813
——◇——	598 nm $y = 23.65 \times 10^{-23}x - 11.93 \times 10^{-22}$	.9774
——△——	617 nm $y = 26.12 \times 10^{-23}x - 5.52 \times 10^{-23}$	.9939
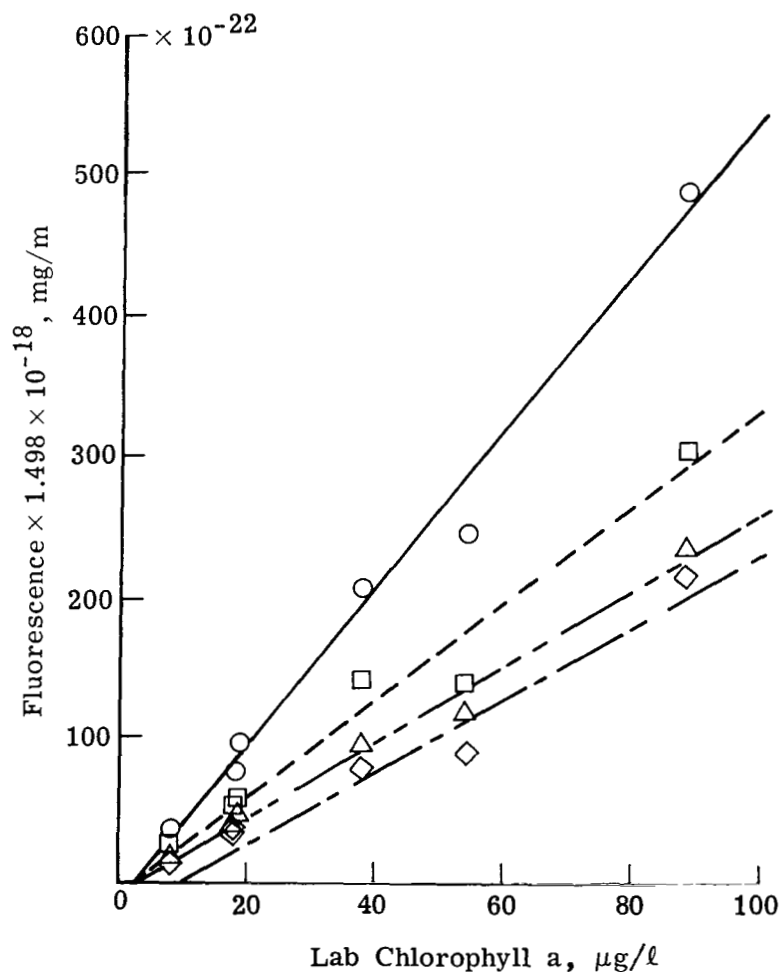
(a) Va-12 species, July 26 to 30, 1976.

Figure 16.- Variation of remote fluorescence with extracted laboratory chlorophyll a for golden-brown algae, Va-12.

			r
—○— 454 nm	$y = 5.14 \times 10^{-22} x + 4.52 \times 10^{-23}$		.9991
---□--- 539 nm	$y = 36.66 \times 10^{-23} x + 13.35 \times 10^{-23}$		.9979
—◇—·— 598 nm	$y = 27.41 \times 10^{-23} x - 5.42 \times 10^{-23}$		.9959
——△--- 617 nm	$y = 26.00 \times 10^{-23} x + 7.58 \times 10^{-23}$		.9983
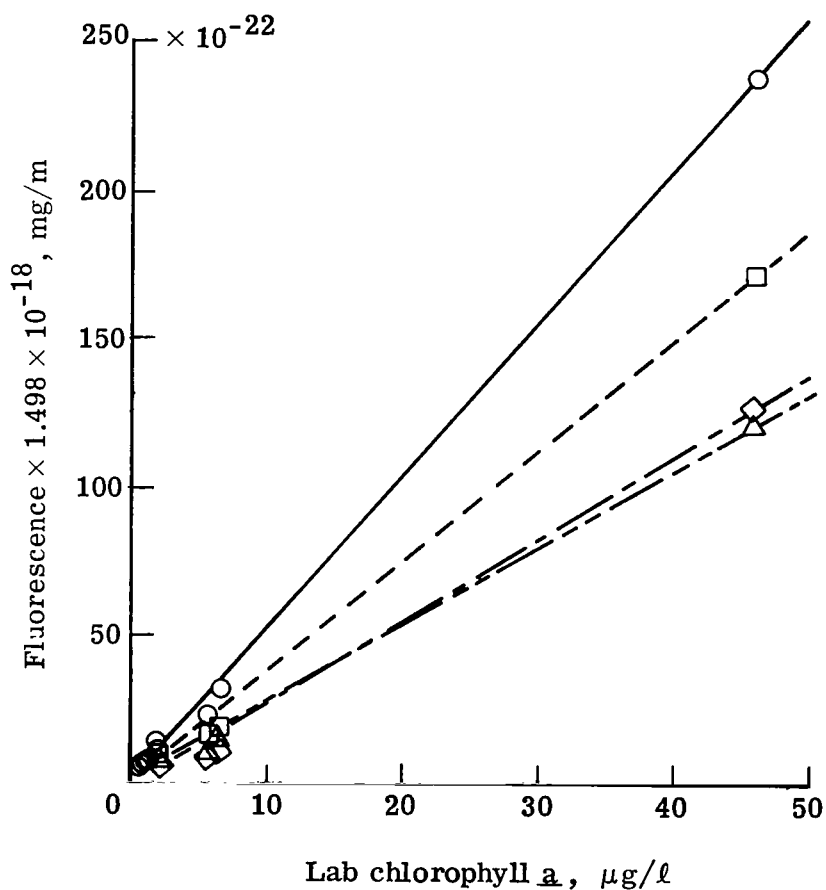
(b) Va-12 species, August 2 to 9, 1976.

Figure 16.- Continued.

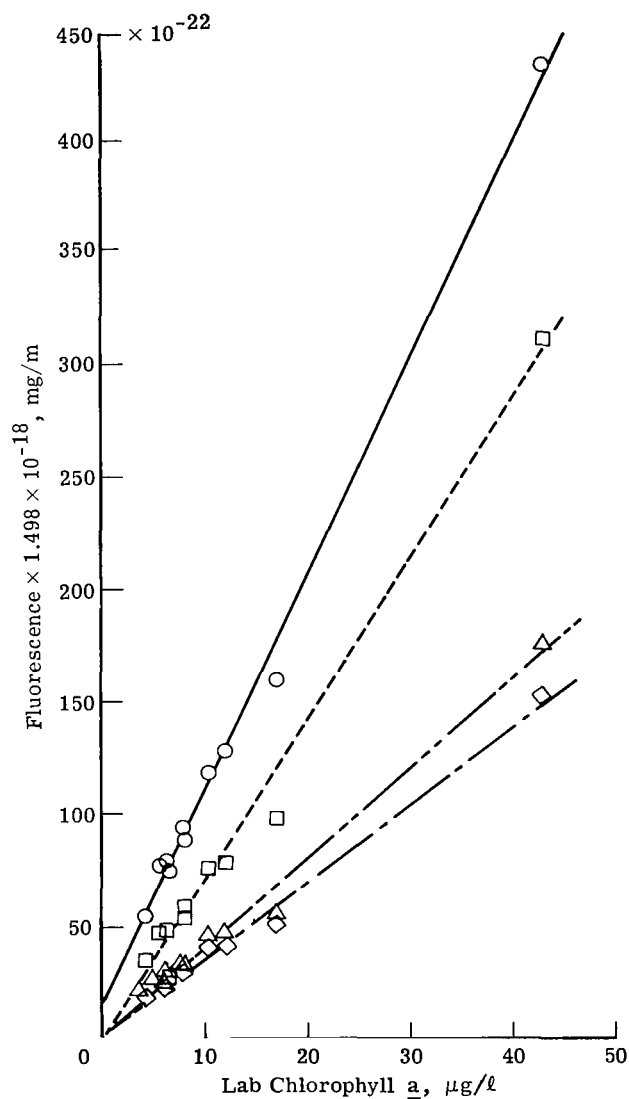|		r
──○── 454 nm	$y = 4.67 \times 10^{-22} x - -4.41 \times 10^{-23}$	.9996
──□── 539 nm	$y = 37.91 \times 10^{-23} x - 14.73 \times 10^{-23}$	.9985
──◇── 598 nm	$y = 18.79 \times 10^{-23} x - 12.25 \times 10^{-24}$	.9987
──△── 617 nm	$y = 28.54 \times 10^{-23} x - 21.19 \times 10^{-23}$	.9981
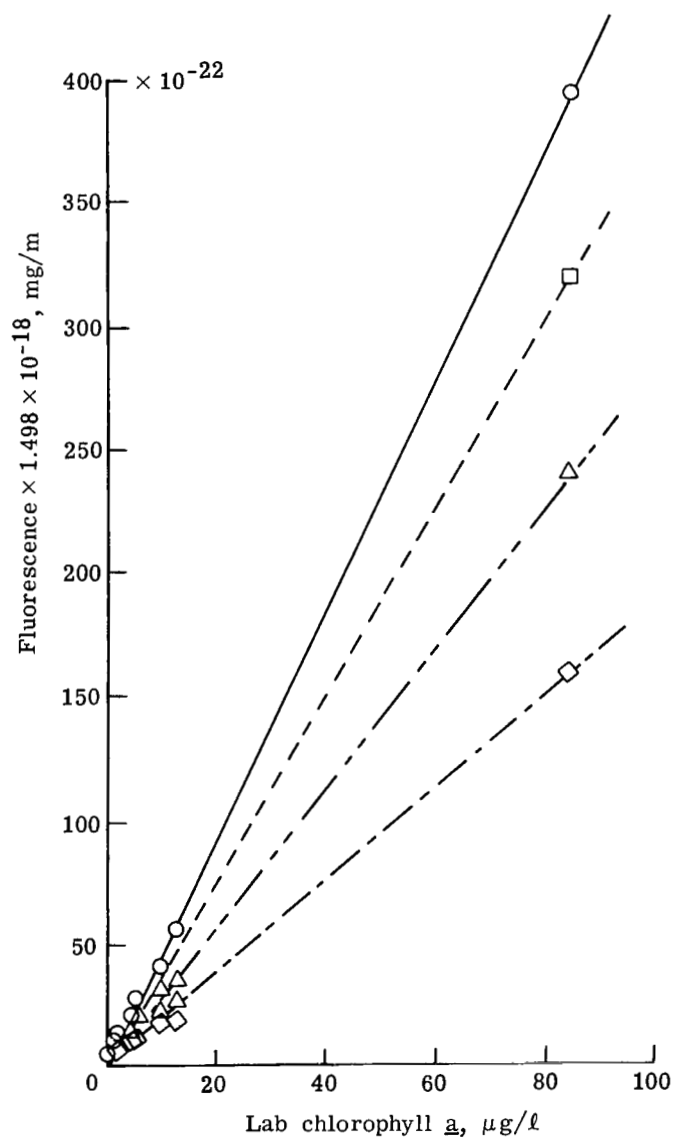
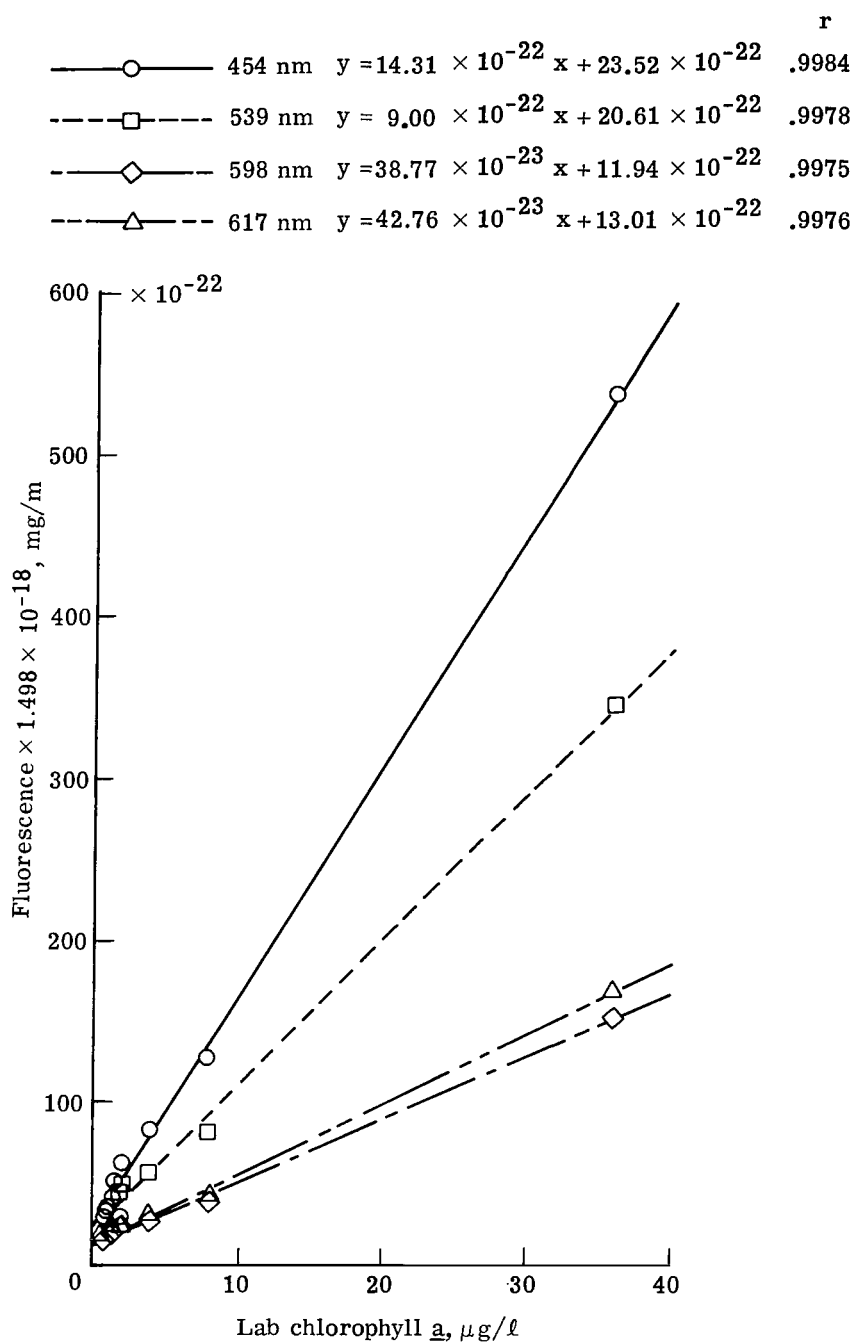(c) Va-12 species, August 9 to 16, 1976.

Figure 16.- Concluded.

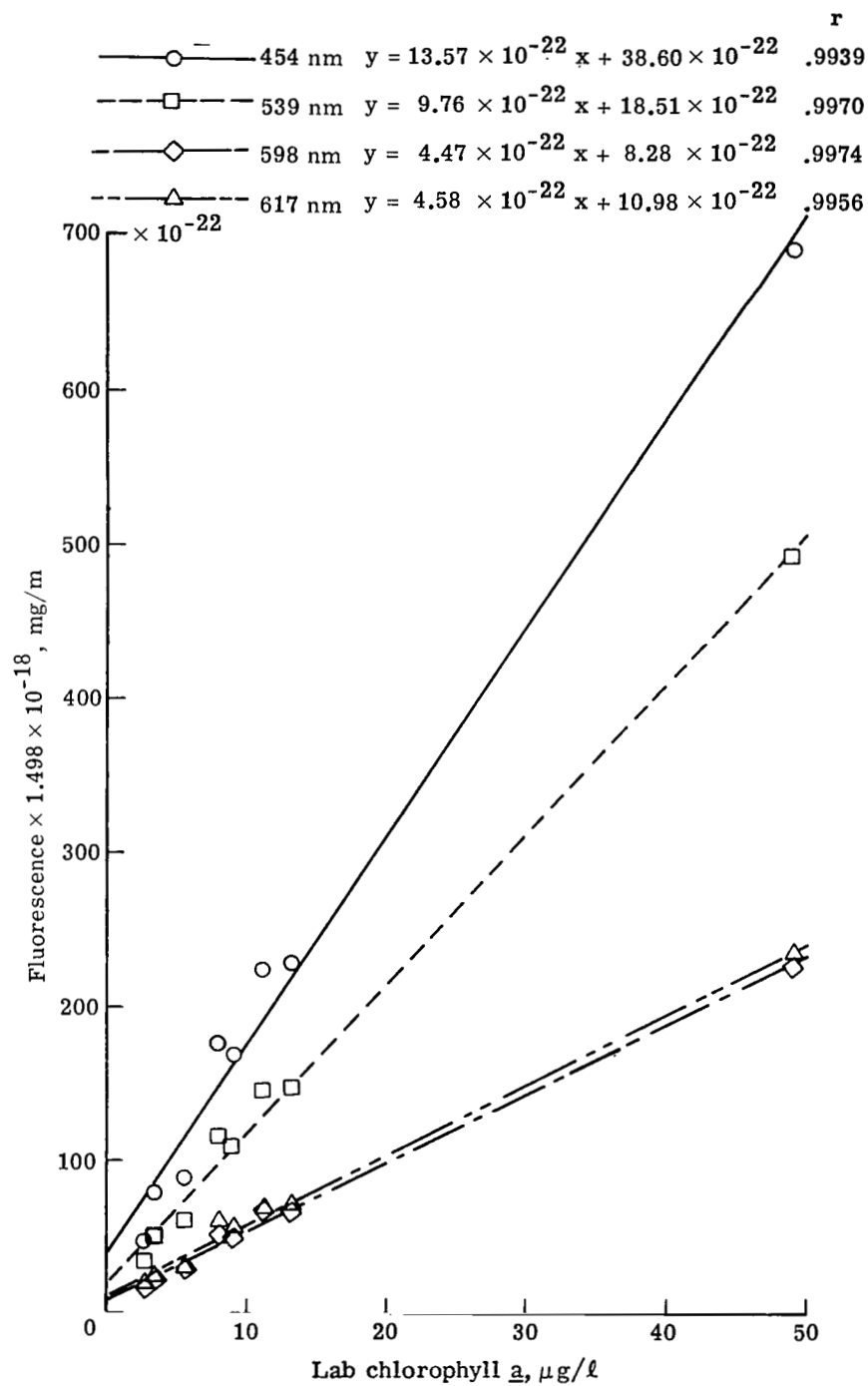|		r
──○── 454 nm	$y = 9.63 \times 10^{-22}x + 13.19 \times 10^{-22}$	.9973
──□── 539 nm	$y = 7.14 \times 10^{-22}x - 31.19 \times 10^{-23}$	.9918
──◇── 598 nm	$y = 34.27 \times 10^{-23}x + 12.88 \times 10^{-23}$	.9945
──△── 617 nm	$y = 39.54 \times 10^{-23}x + 13.27 \times 10^{-23}$	.9928

(a) Va-13 species, July 9 to 19, 1976.

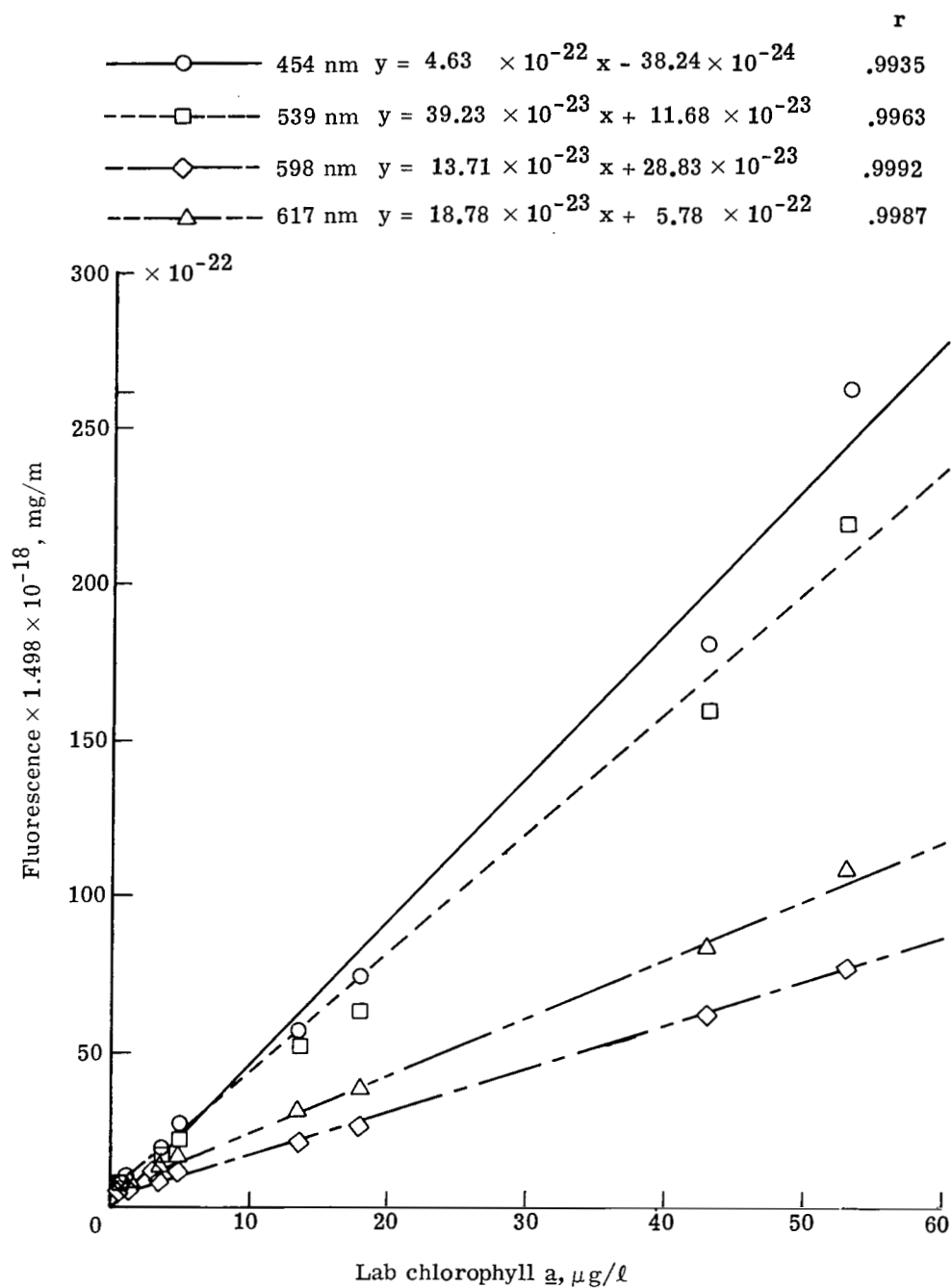Figure 17.- Variation of remote fluorescence with extracted laboratory chlorophyll a for dinoflagellate algae, Va-13.

		r
—○— 454 nm	$y = 14.31 \times 10^{-22} x + 23.52 \times 10^{-22}$	.9984
—□— 539 nm	$y = 9.00 \times 10^{-22} x + 20.61 \times 10^{-22}$	.9978
—◇— 598 nm	$y = 38.77 \times 10^{-23} x + 11.94 \times 10^{-22}$	.9975
—△— 617 nm	$y = 42.76 \times 10^{-23} x + 13.01 \times 10^{-22}$	.9976

Fluorescence $\times 1.498 \times 10^{-18}$, mg/m

Lab chlorophyll a, $\mu g/\ell$

(b) Va-13 species, August 27 to September 7, 1976.

Figure 17.- Continued.

		r
—○— — 454 nm	$y = 13.57 \times 10^{-22}\, x + 38.60 \times 10^{-22}$	.9939
—□— — 539 nm	$y = 9.76 \times 10^{-22}\, x + 18.51 \times 10^{-22}$	.9970
—◇— — 598 nm	$y = 4.47 \times 10^{-22}\, x + 8.28 \times 10^{-22}$	.9974
—△— — 617 nm	$y = 4.58 \times 10^{-22}\, x + 10.98 \times 10^{-22}$	.9956

Fluorescence $\times 1.498 \times 10^{-18}$, mg/m

$\times 10^{-22}$

700

600

500

400

300

200

100

0   10   20   30   40   50

Lab chlorophyll a, μg/ℓ

(c) Va-13 species, September 13 to 20, 1976.

Figure 17.- Concluded.

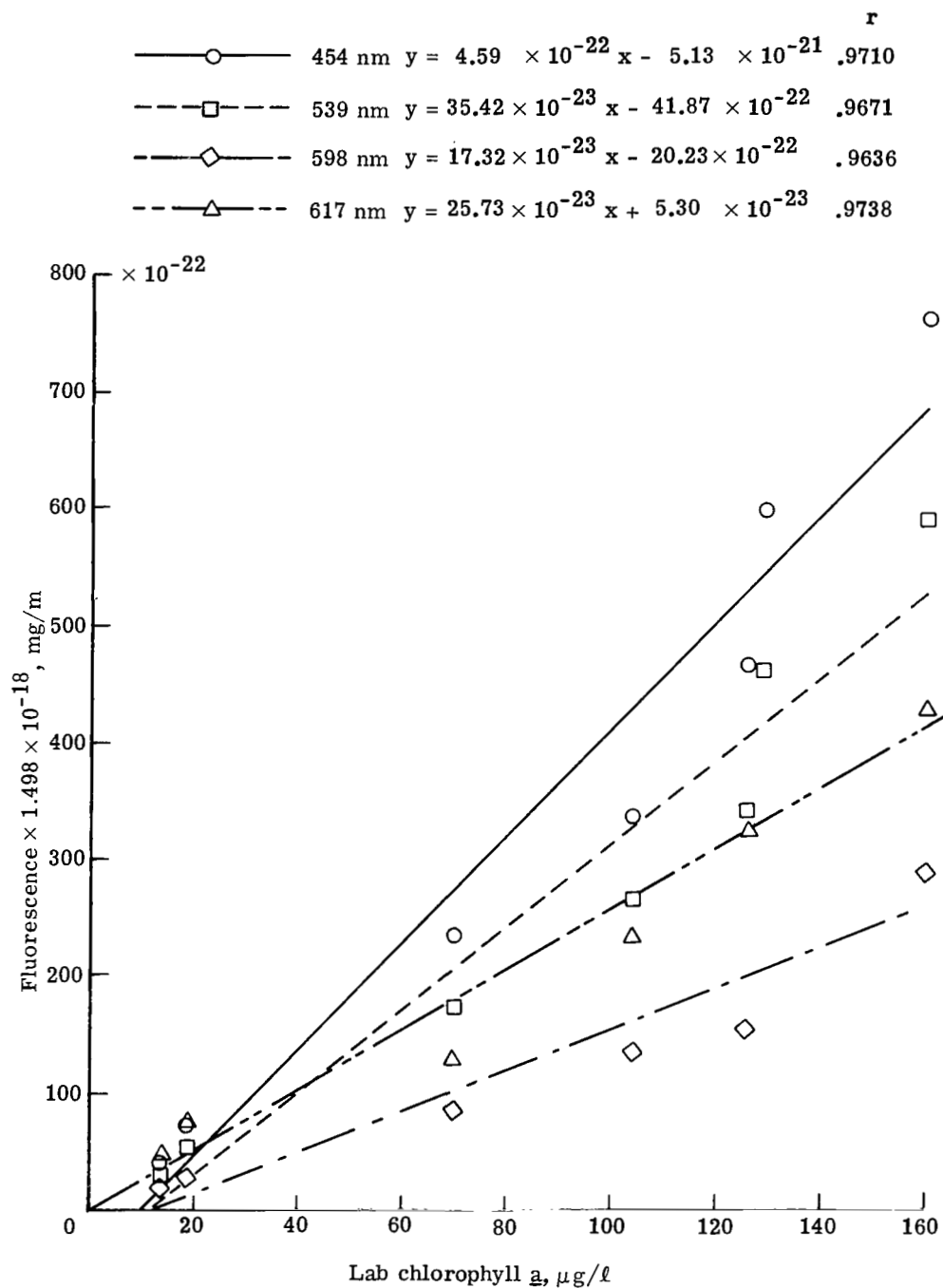|			r
——○——	454 nm	$y = 4.63 \times 10^{-22} x - 38.24 \times 10^{-24}$	.9935
——□——	539 nm	$y = 39.23 \times 10^{-23} x + 11.68 \times 10^{-23}$	.9963
——◇——	598 nm	$y = 13.71 \times 10^{-23} x + 28.83 \times 10^{-23}$	.9992
——△——	617 nm	$y = 18.78 \times 10^{-23} x + 5.78 \times 10^{-22}$	.9987

(a) Va-72 species, October 5 to 8, 1976.

Figure 18.- Variation of remote fluorescence with extracted laboratory chlorophyll a for diatom algae, Va-72.
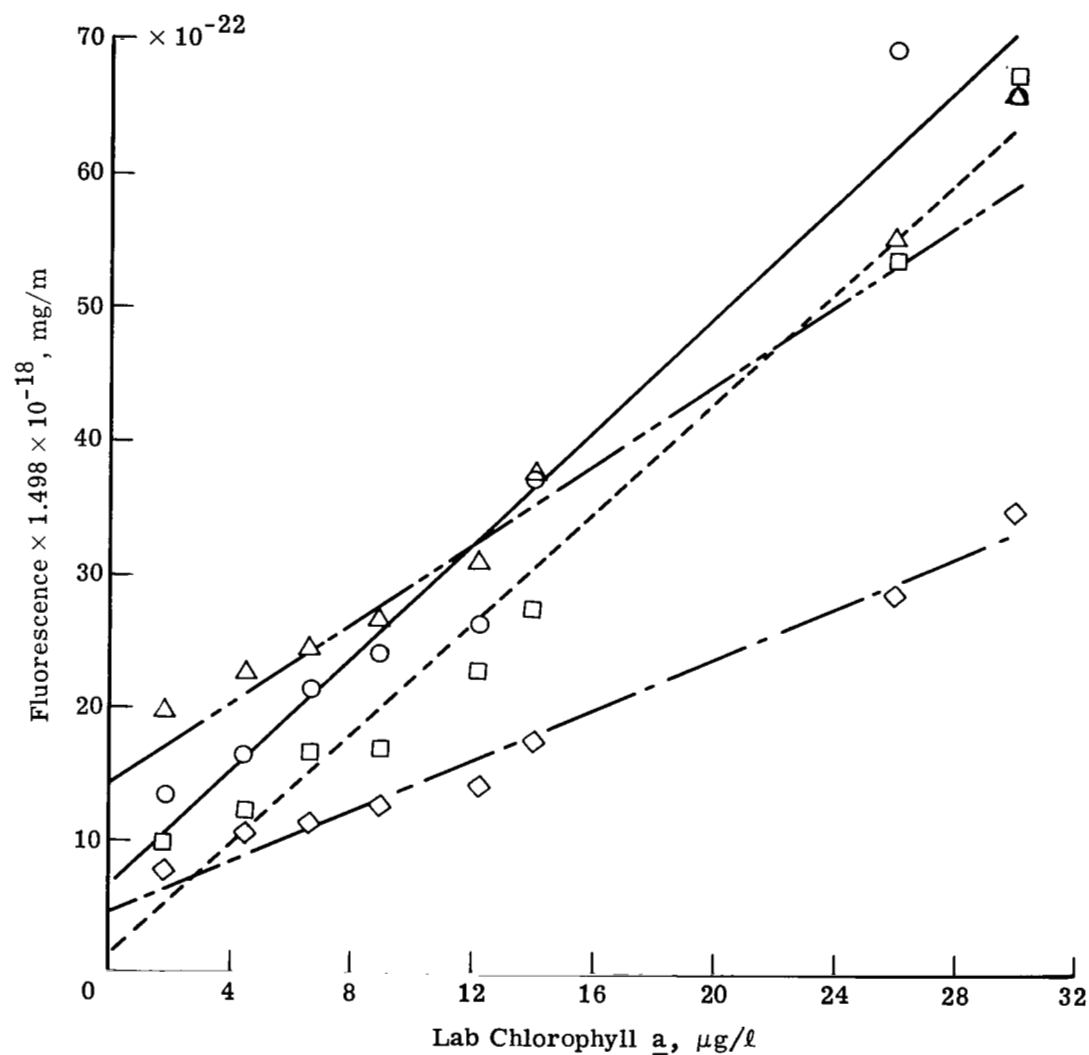
58

(b) Va-72 species, October 12 to 19, 1976.
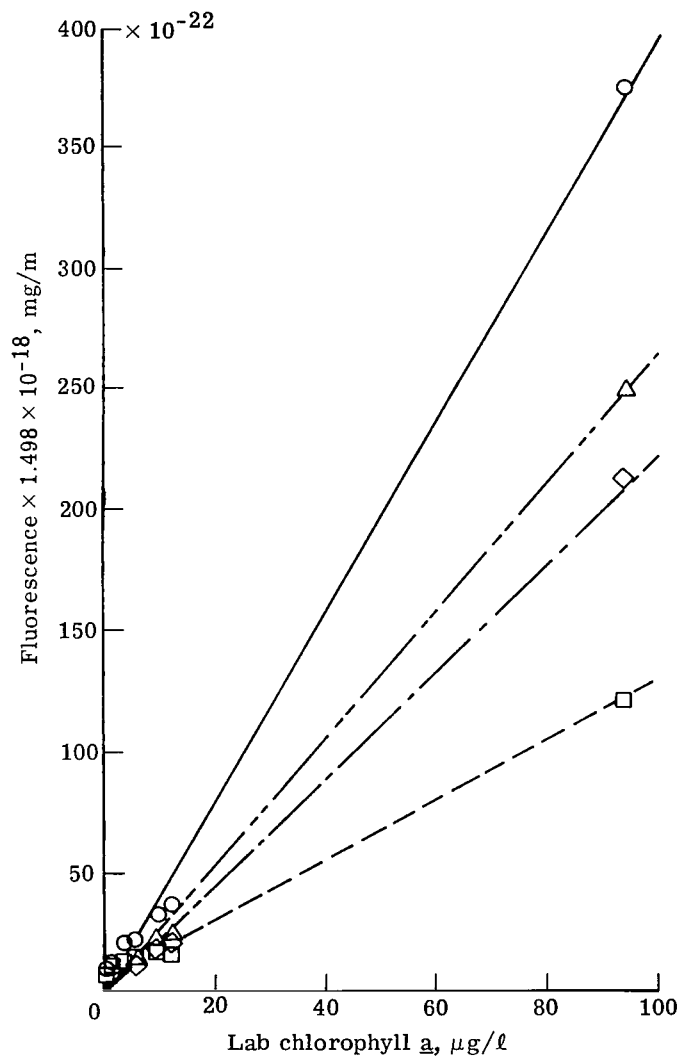
Figure 18.- Continued.

The legend at top right of the figure reads:

		r
454 nm	$y = 4.59 \times 10^{-22} x - 5.13 \times 10^{-21}$	.9710
539 nm	$y = 35.42 \times 10^{-23} x - 41.87 \times 10^{-22}$	.9671
598 nm	$y = 17.32 \times 10^{-23} x - 20.23 \times 10^{-22}$	.9636
617 nm	$y = 25.73 \times 10^{-23} x + 5.30 \times 10^{-23}$	.9738

Fluorescence $\times\ 1.498 \times 10^{-18}$, mg/m

Lab chlorophyll $\underline{a}$, $\mu g/\ell$

(c) Va-72 species, January 28 to February 3, 1977.

Figure 18.- Continued.

(d) Va-72 species, June 28 to July 2, 1977.

Figure 18.- Concluded.

$$\text{454 nm} \quad y = 15.80 \times 10^{-23}\,x + 7.73 \times 10^{-22} \quad .9923$$

$$\text{539 nm} \quad y = 20.72 \times 10^{-23}\,x + 6.67 \times 10^{-22} \quad .9792$$

$$\text{598 nm} \quad y = 8.94 \times 10^{-23}\,x + 34.78 \times 10^{-23} \quad .9925$$

$$\text{617 nm} \quad y = 11.99 \times 10^{-23}\,x + 35.80 \times 10^{-23} \quad .9928$$
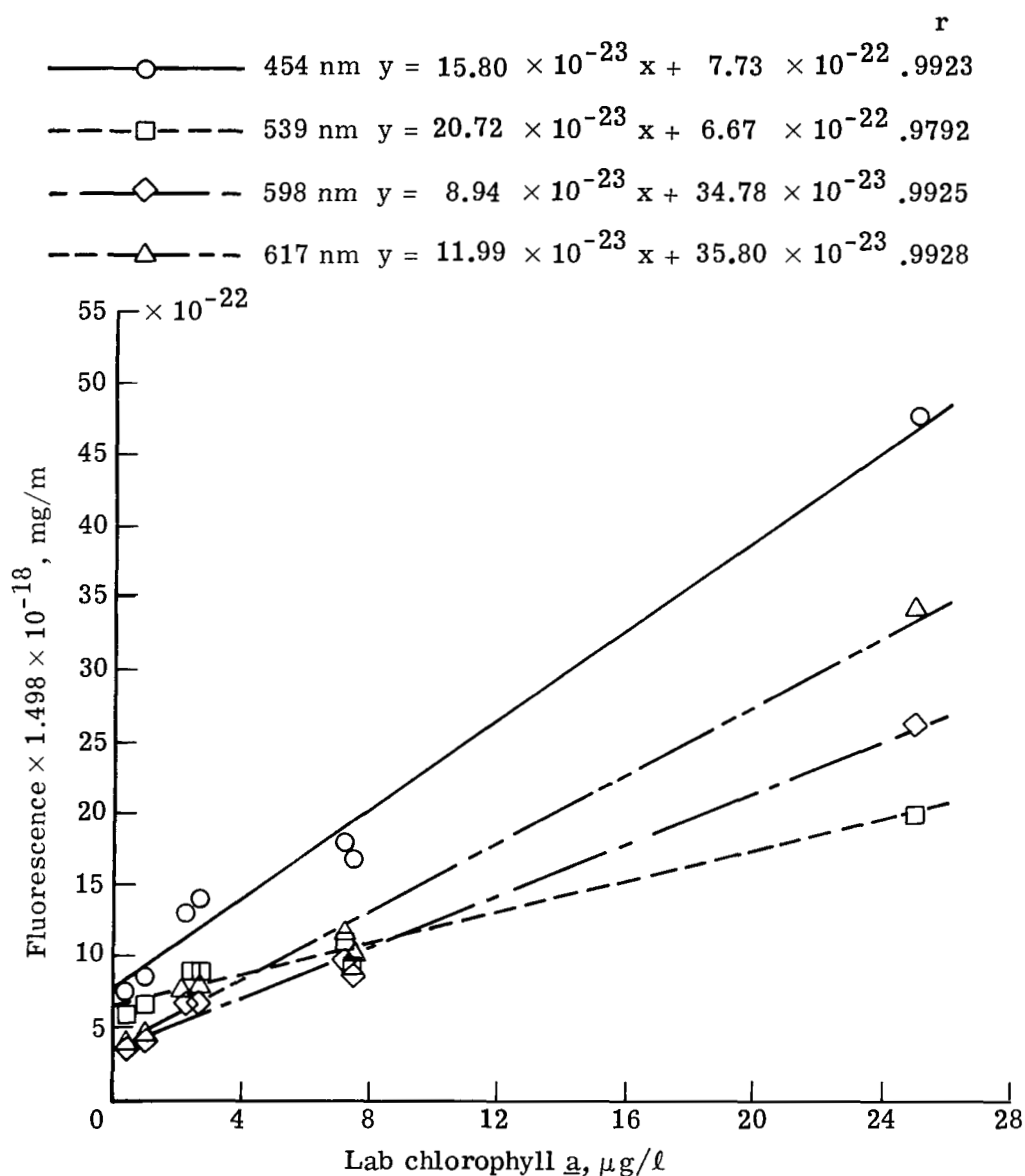
(a) Va-74 species, September 20 to 27, 1976.

Figure 19.- Variation of remote fluorescence with extracted laboratory chlorophyll a for green algae, Va-74.
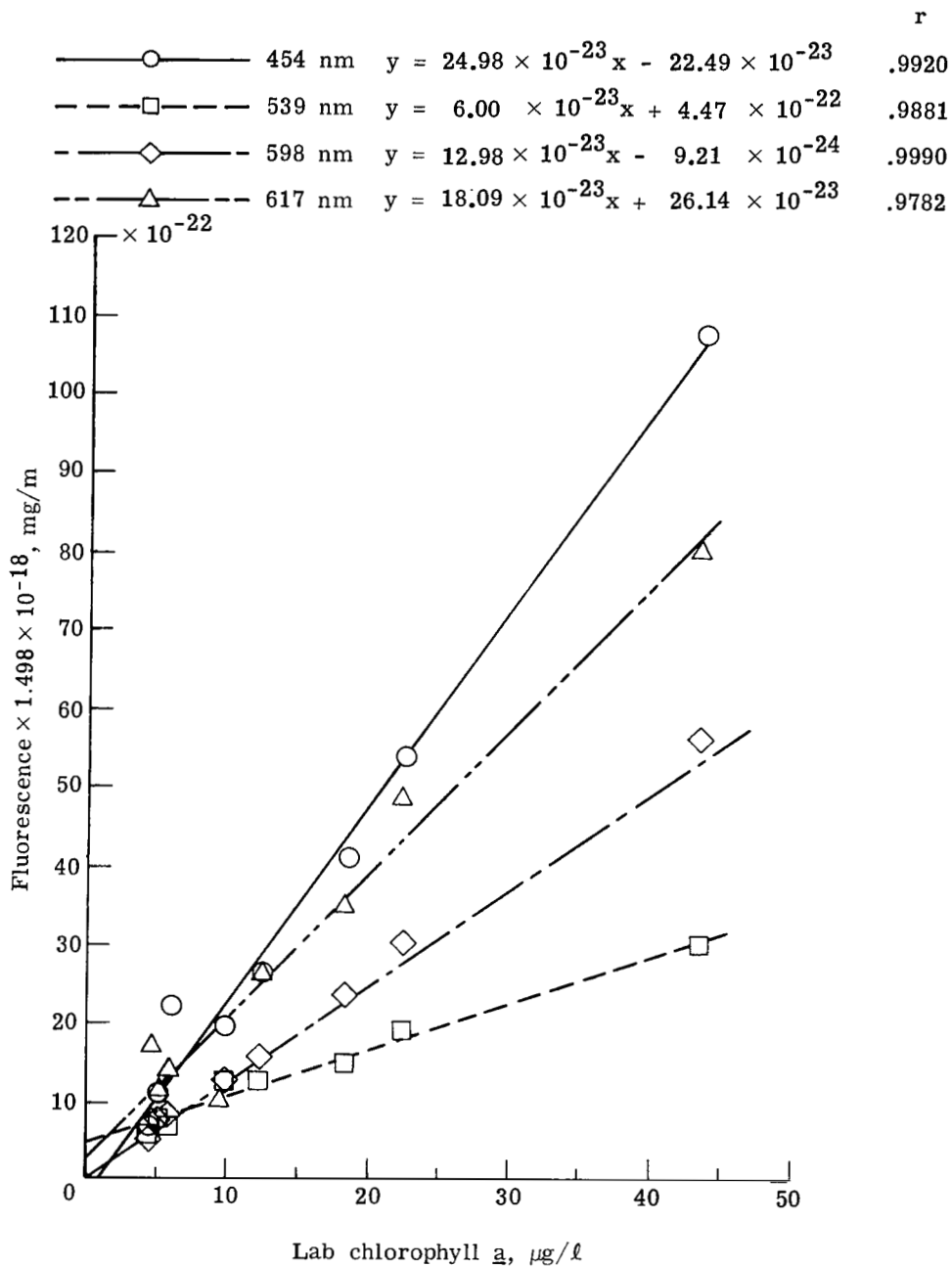
(b) Va-74 species, September 27 to October 4, 1976.

Figure 19.- Continued.

				r
○	454 nm	$y = 24.98 \times 10^{-23}x - 22.49 \times 10^{-23}$		.9920
□	539 nm	$y = 6.00 \times 10^{-23}x + 4.47 \times 10^{-22}$		.9881
◇	598 nm	$y = 12.98 \times 10^{-23}x - 9.21 \times 10^{-24}$		.9990
△	617 nm	$y = 18.09 \times 10^{-23}x + 26.14 \times 10^{-23}$		.9782

Fluorescence $\times 1.498 \times 10^{-18}$, mg/m

Lab chlorophyll a, $\mu g/\ell$

(c) Va-74 species, November 1 to 6, 1976.

Figure 19.- Continued.

$$
\begin{array}{llll}
\underline{\quad\circ\quad} & 454 \text{ nm} & y = 30.81 \times 10^{-23} \, x - 30.66 \times 10^{-22} & .9970 \\
\underline{--\square--} & 539 \text{ nm} & y = 10.17 \times 10^{-23} \, x - 25.56 \times 10^{-22} & .9865 \\
\underline{--\diamond--} & 598 \text{ nm} & y = 24.41 \times 10^{-23} \, x - 5.74 \times 10^{-21} & .9885 \\
\underline{--\triangle--} & 617 \text{ nm} & y = 31.18 \times 10^{-23} \, x - 5.50 \times 10^{-21} & .9918 \\
\end{array}
$$
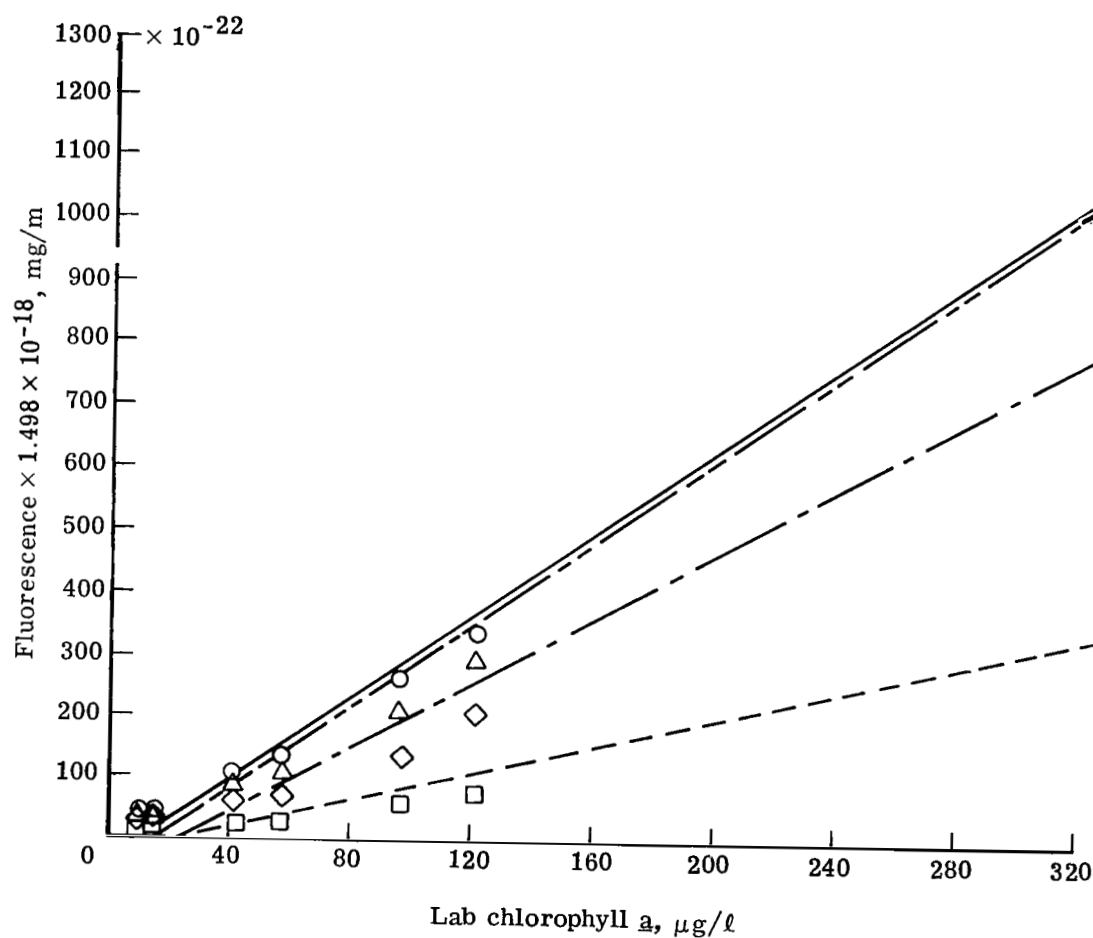
(d) Va-74 species, June 22 to 27, 1977.

Figure 19.- Concluded.

$$y = 6.72 \times 10^{-23}x + 12.01 \times 10^{-22} \qquad r$$

──◯──	454 nm	$y = 6.72 \times 10^{-23}x + 12.01 \times 10^{-22}$	.9847
──□──	539 nm	$y = 32.18 \times 10^{-23}x + 10.57 \times 10^{-22}$	.9861
──◇──	598 nm	$y = 10.72 \times 10^{-23}x + 8.98 \times 10^{-22}$	.9900
──△──	617 nm	$y = 12.69 \times 10^{-23}x + 19.21 \times 10^{-23}$	.9728
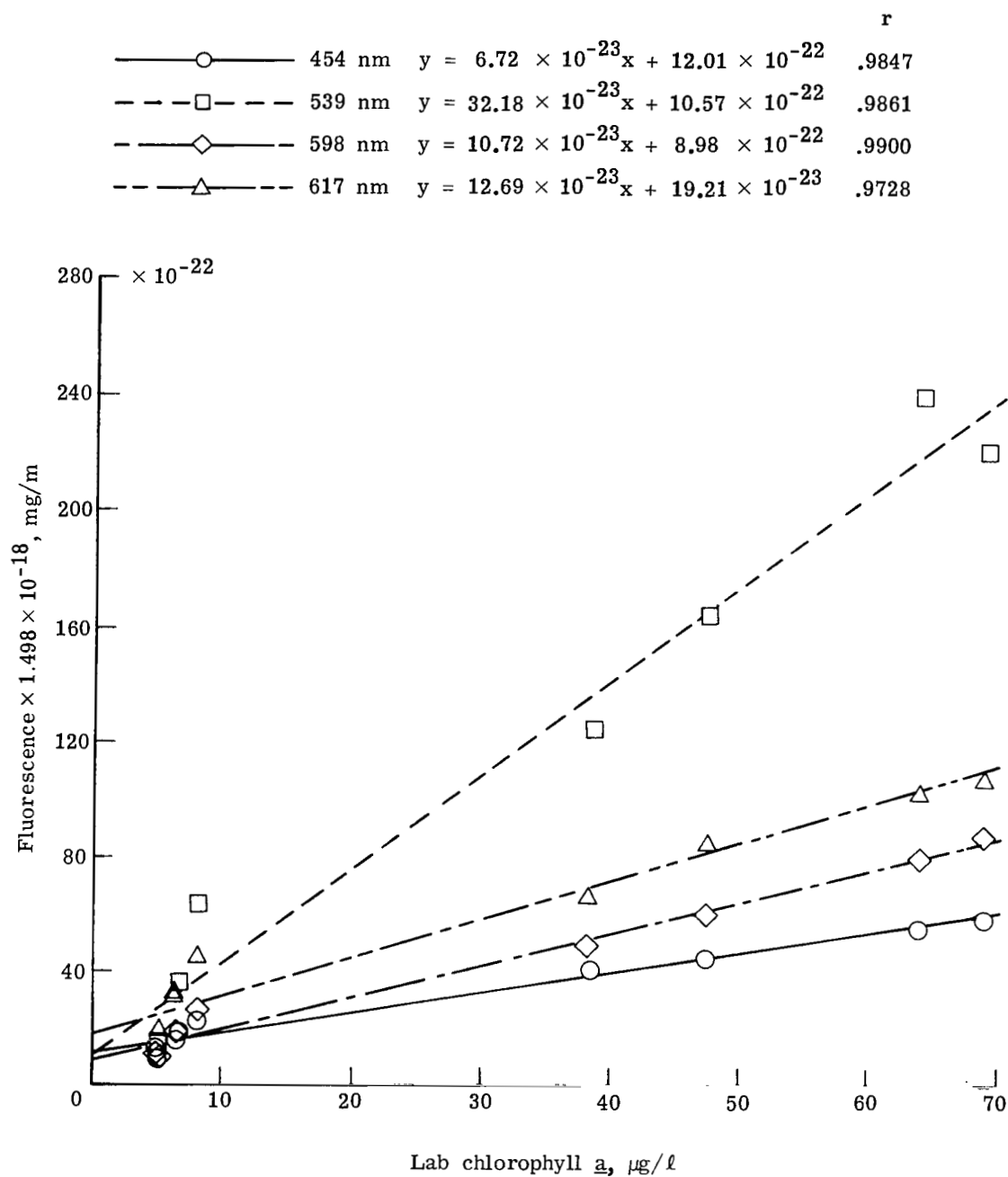
(a) Va-70 species, November 17 to 23, 1976.

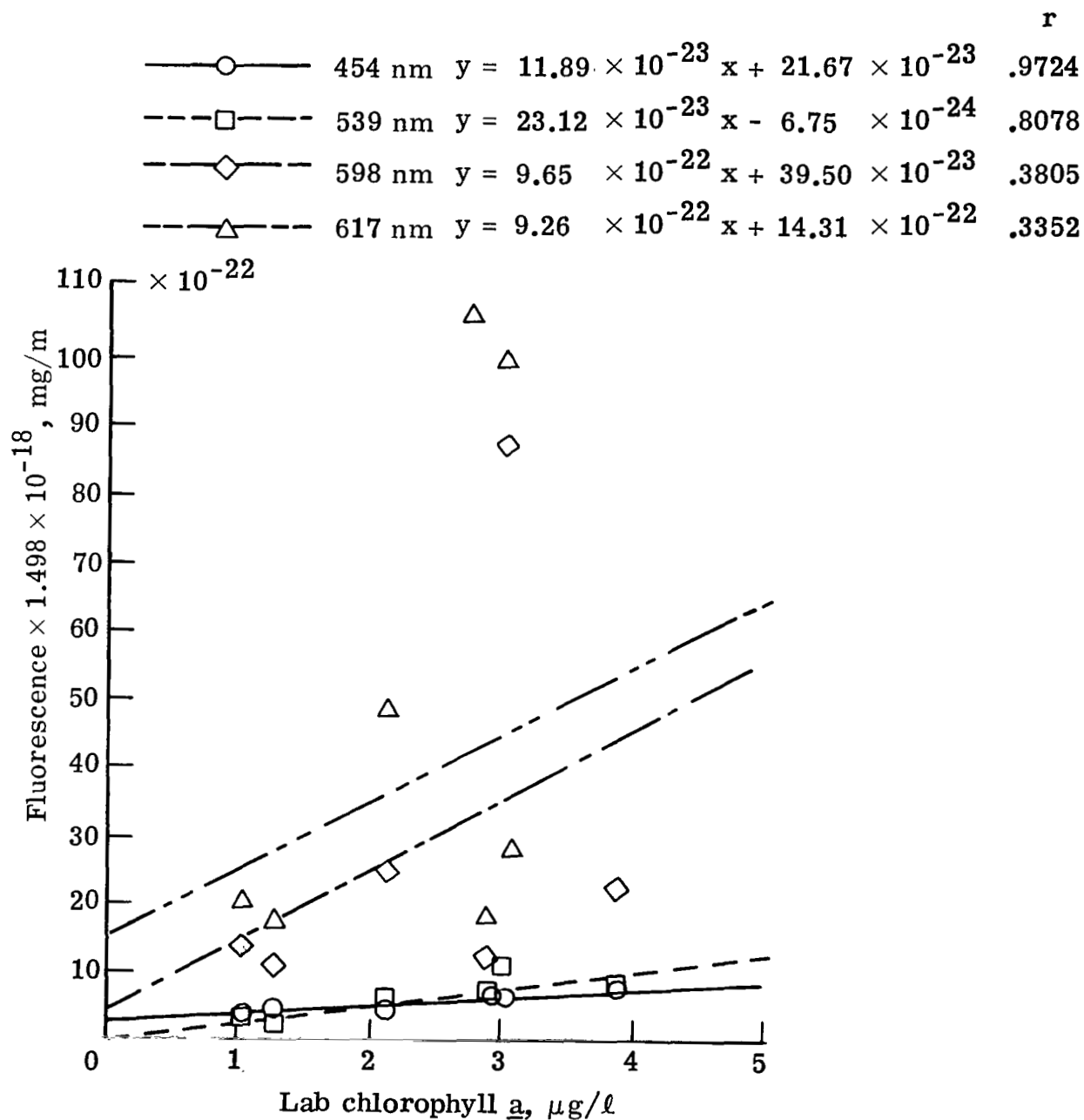Figure 20.- Variation of remote fluorescence with extracted laboratory chlorophyll a for red algae, Va-70.
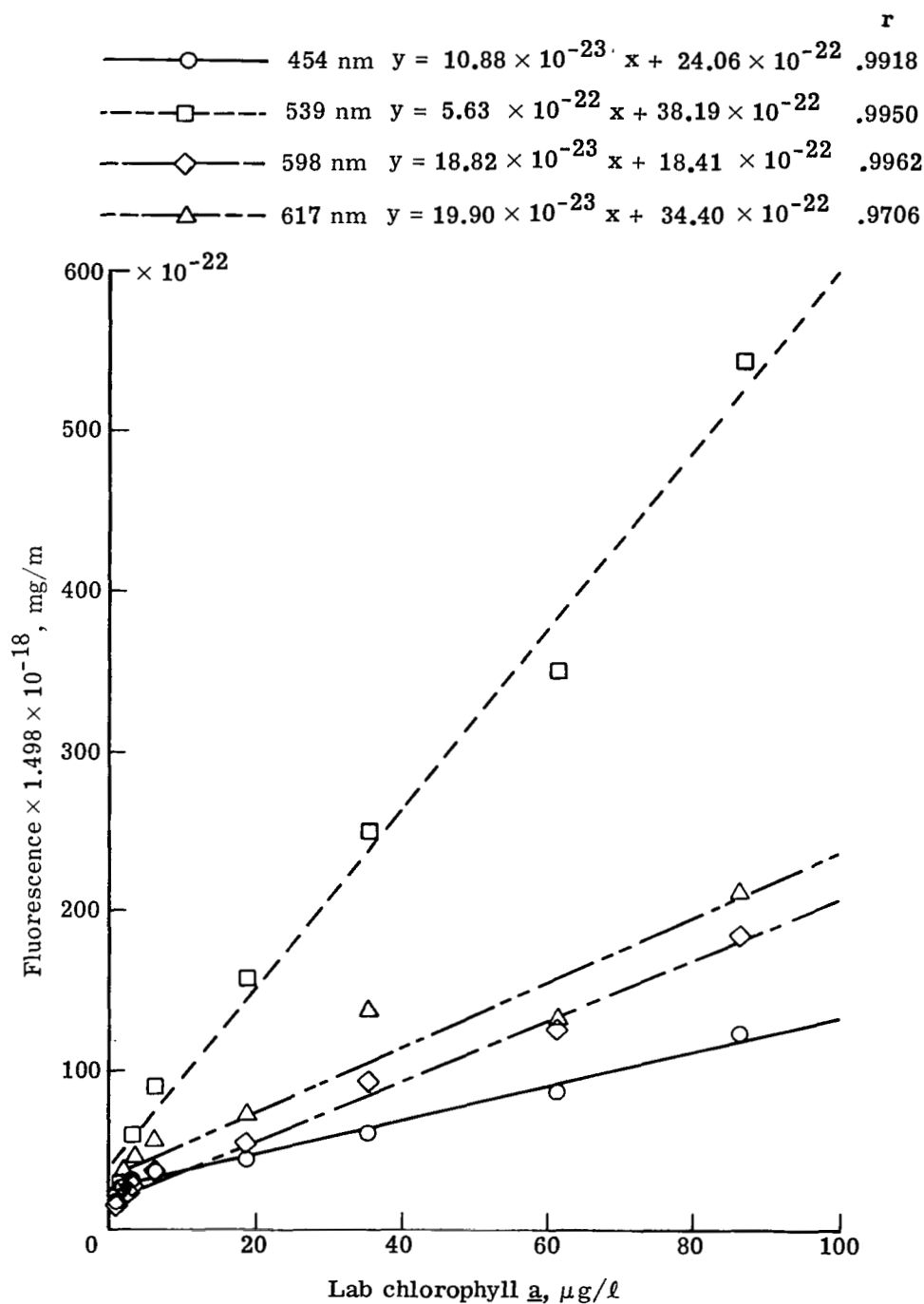
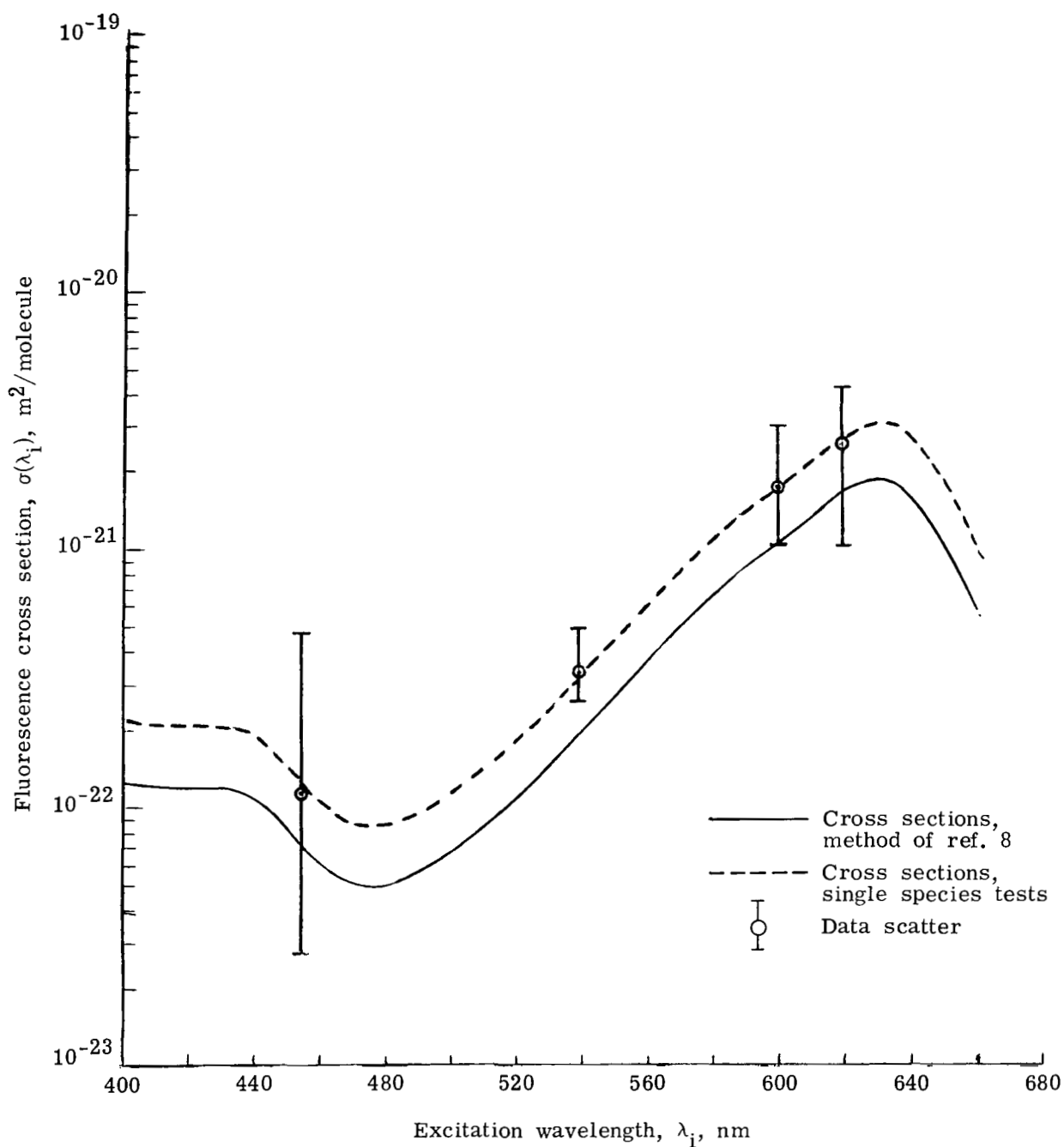(b) Va-70 species, March 28 to April 7, 1977.

Figure 20.- Concluded.

The legend shows:

454 nm $y = 11.34 \times 10^{-23}x + 37.39 \times 10^{-22}$, $r = .7012$

539 nm $y = 4.44 \times 10^{-22}x + 37.48 \times 10^{-22}$, $r = .9399$

598 nm $y = 25.07 \times 10^{-22}x - 5.04 \times 10^{-22}$, $r = .9788$

617 nm $y = 41.08 \times 10^{-22}x + 12.23 \times 10^{-21}$, $r = .9724$
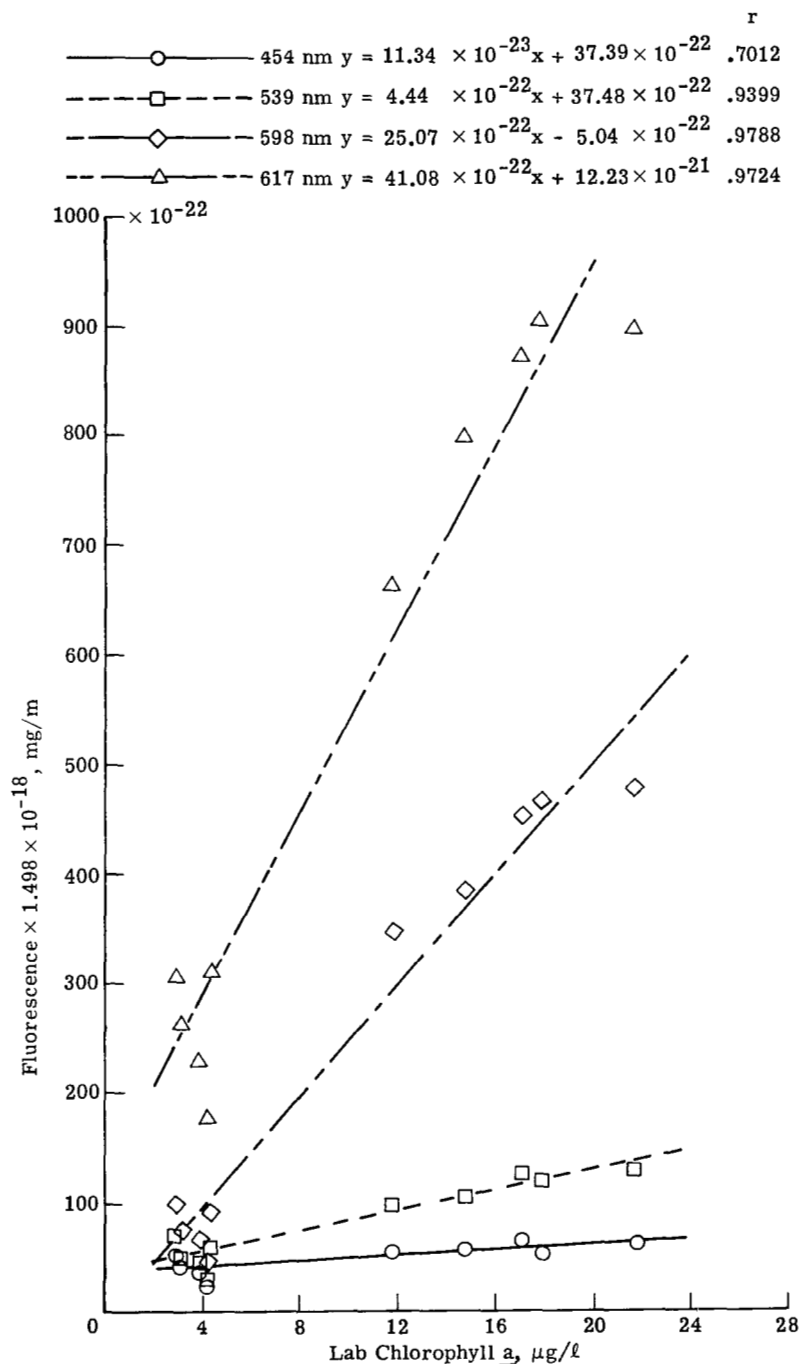
(a) Va-9 species, February 25 to March 4, 1977.

Figure 21.- Variation of remote fluorescence with extracted laboratory chlorophyll a for blue-green algae, Va-9.

$$r$$

$$\text{454 nm} \quad y = 11.89 \times 10^{-23} x + 21.67 \times 10^{-23} \quad .9724$$

$$\text{539 nm} \quad y = 23.12 \times 10^{-23} x - 6.75 \times 10^{-24} \quad .8078$$

$$\text{598 nm} \quad y = 9.65 \times 10^{-22} x + 39.50 \times 10^{-23} \quad .3805$$

$$\text{617 nm} \quad y = 9.26 \times 10^{-22} x + 14.31 \times 10^{-22} \quad .3352$$

(b) Va-9 species, April 25 to May 3, 1977.

Figure 21.- Concluded.

(a) Blue-green algae, Va-9.

Figure 22.- Comparison of shapes of curves for fluorescence cross sections
determined by a fluorescence spectrophotometer with averaged values
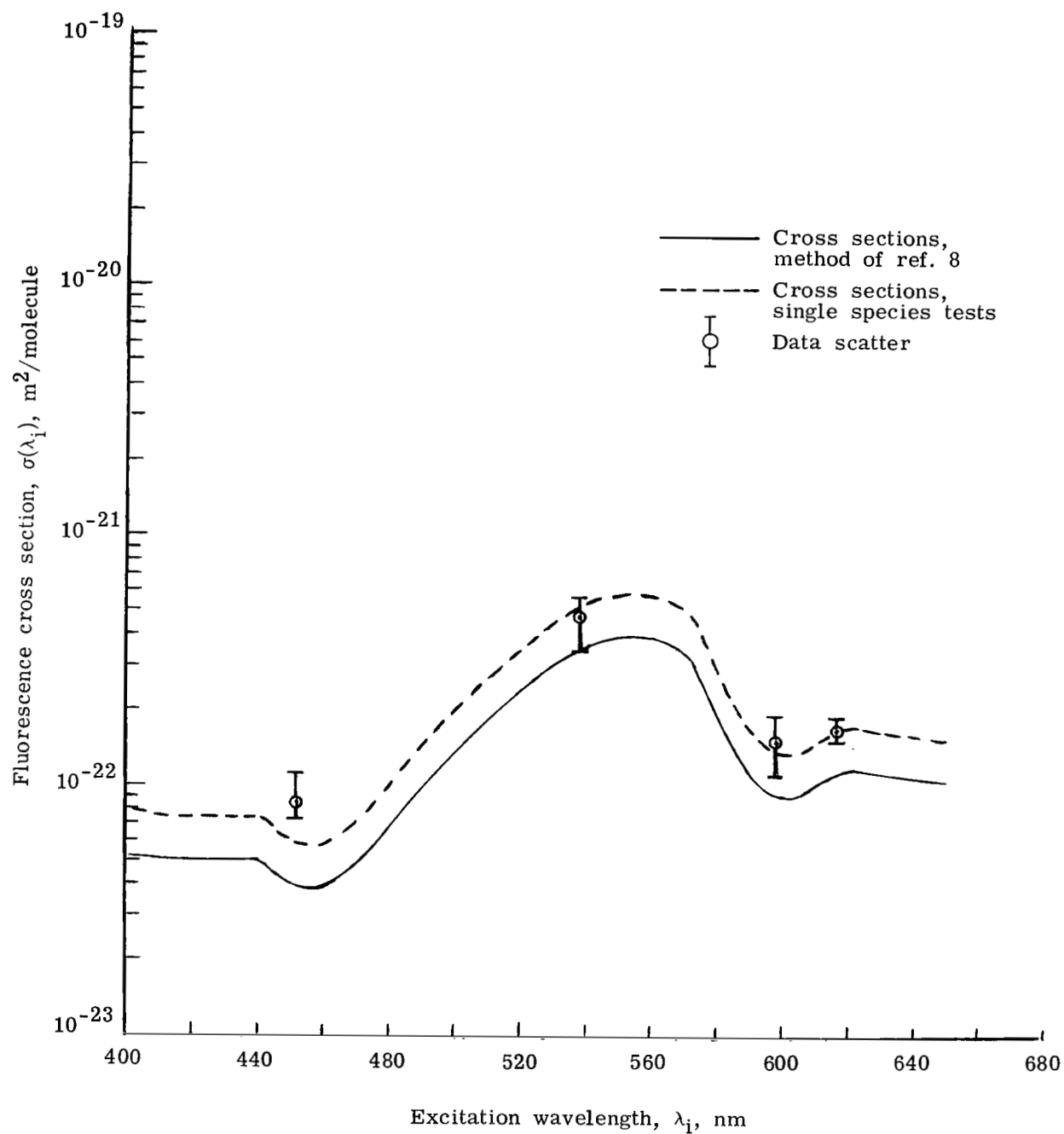determined from single species tank tests.
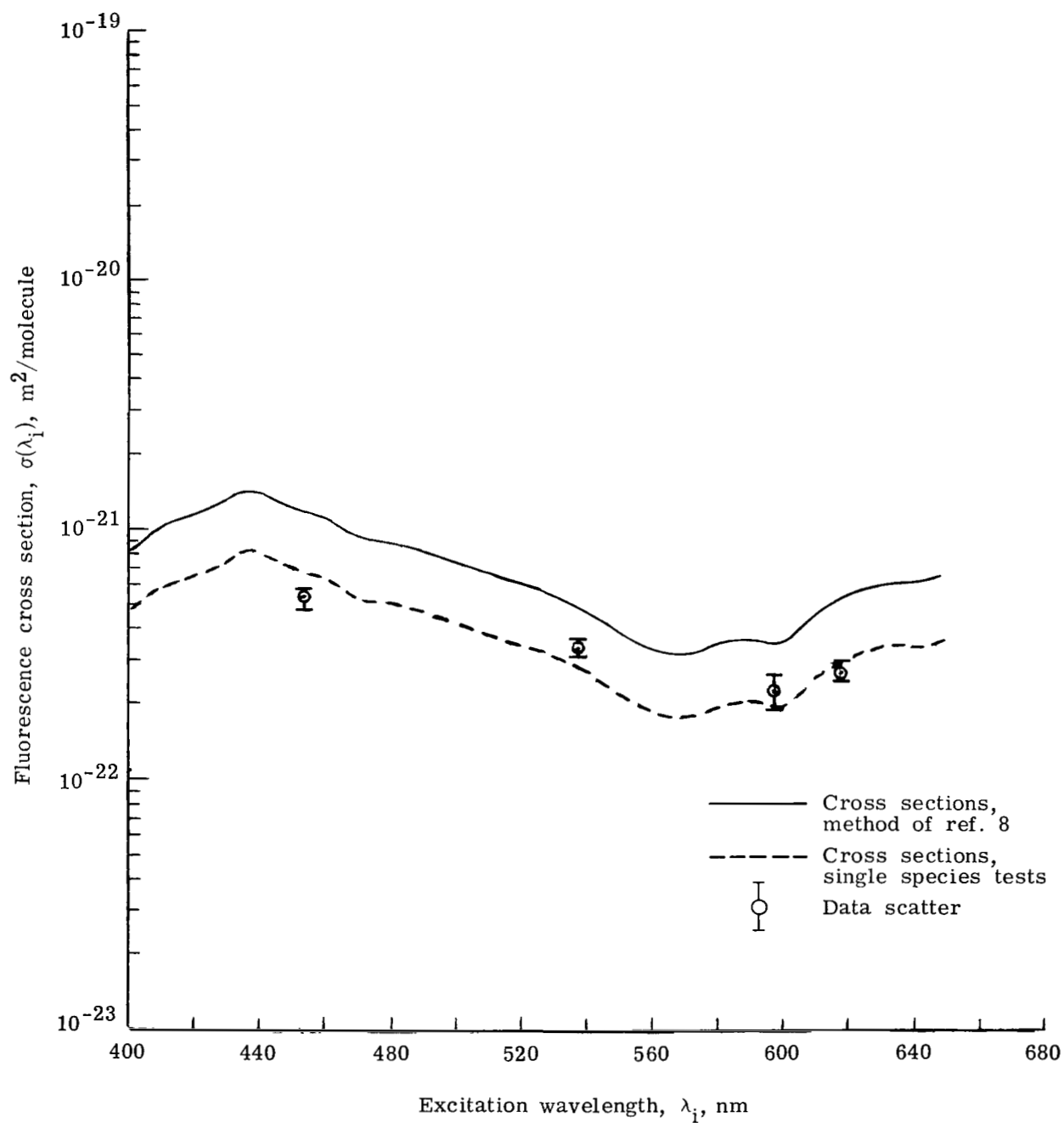
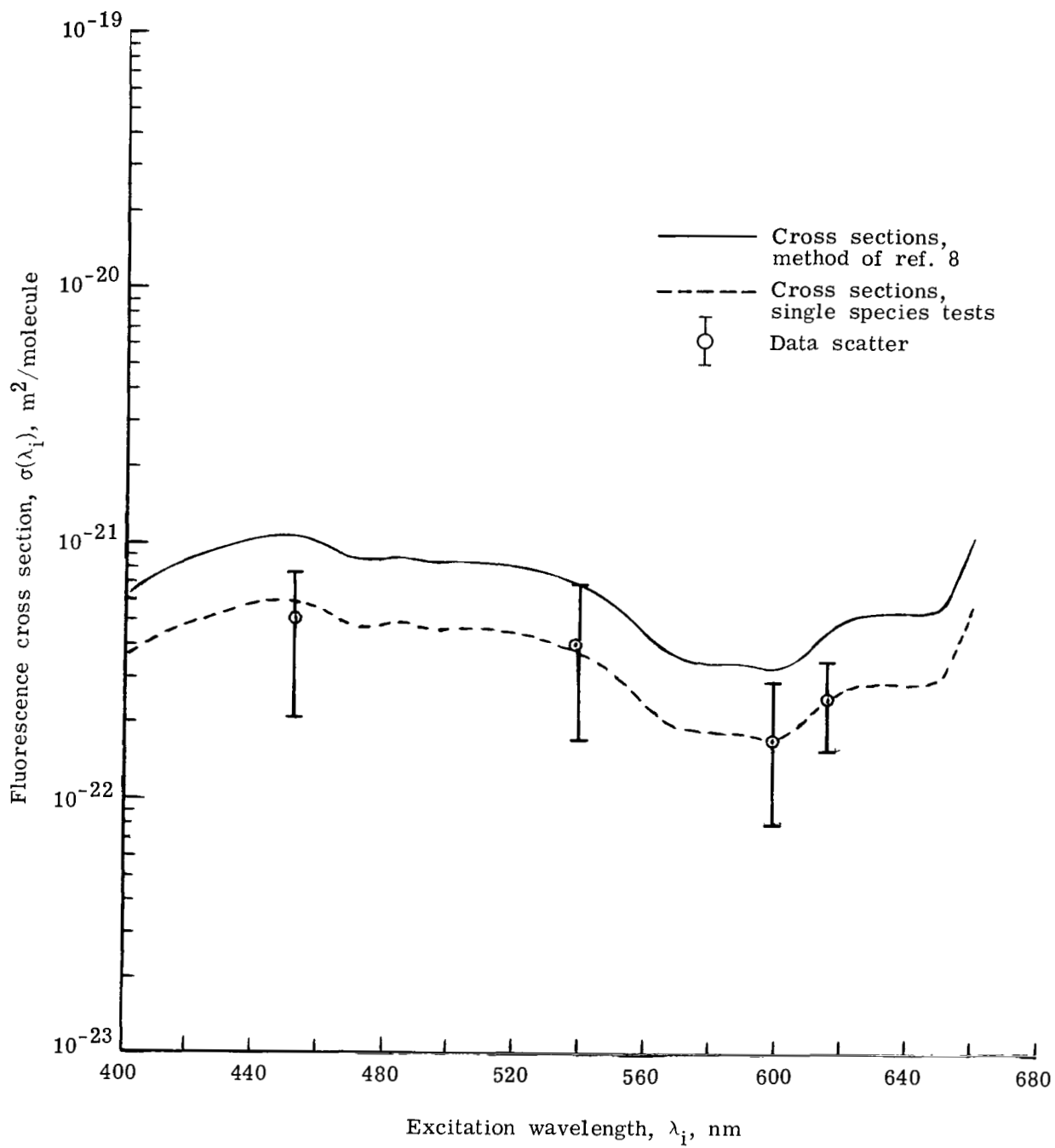(b) Yellow-green algae, Va-12.

Figure 22.- Continued.
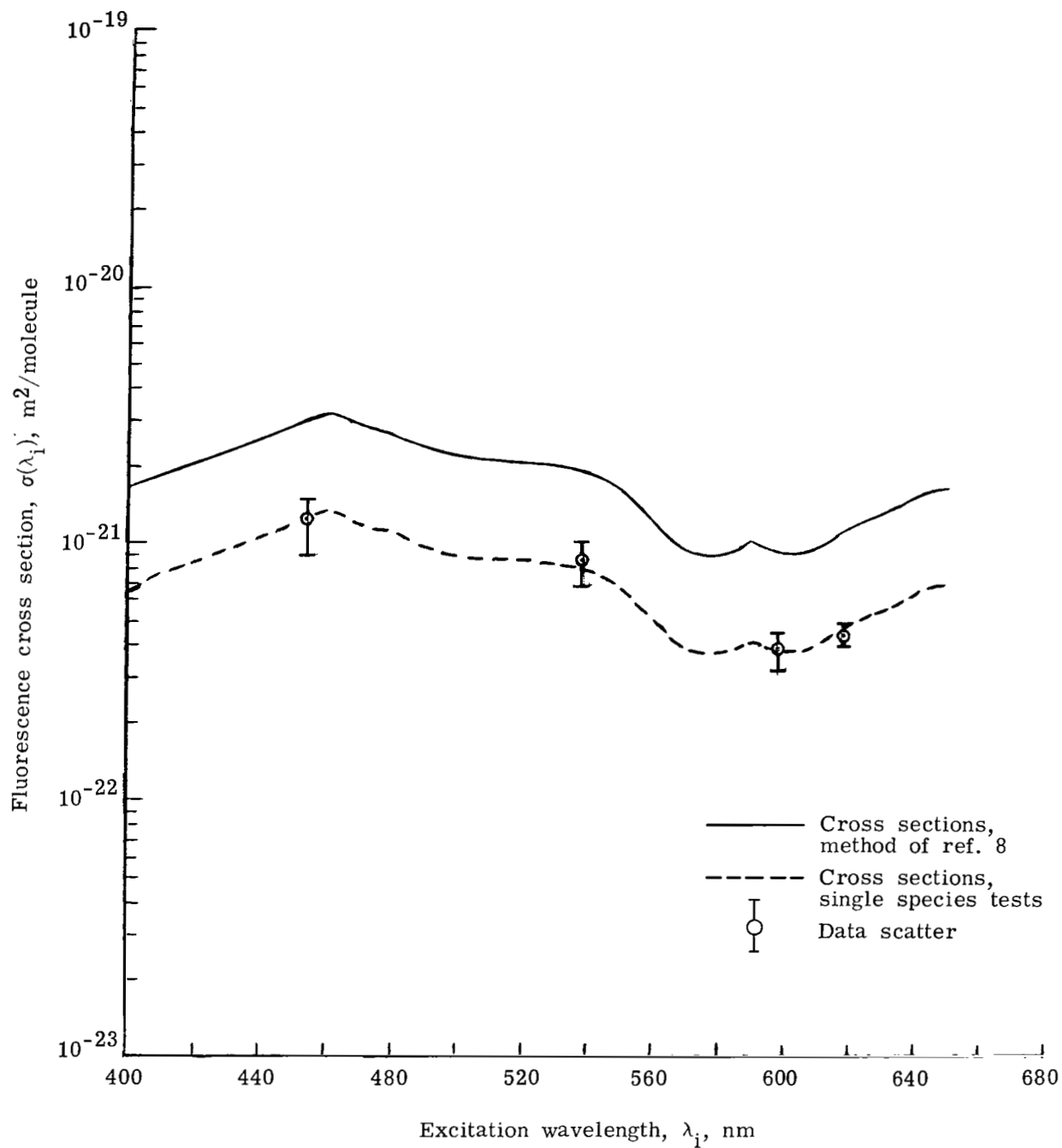
(c) Dinoflagellate algae, Va-13.
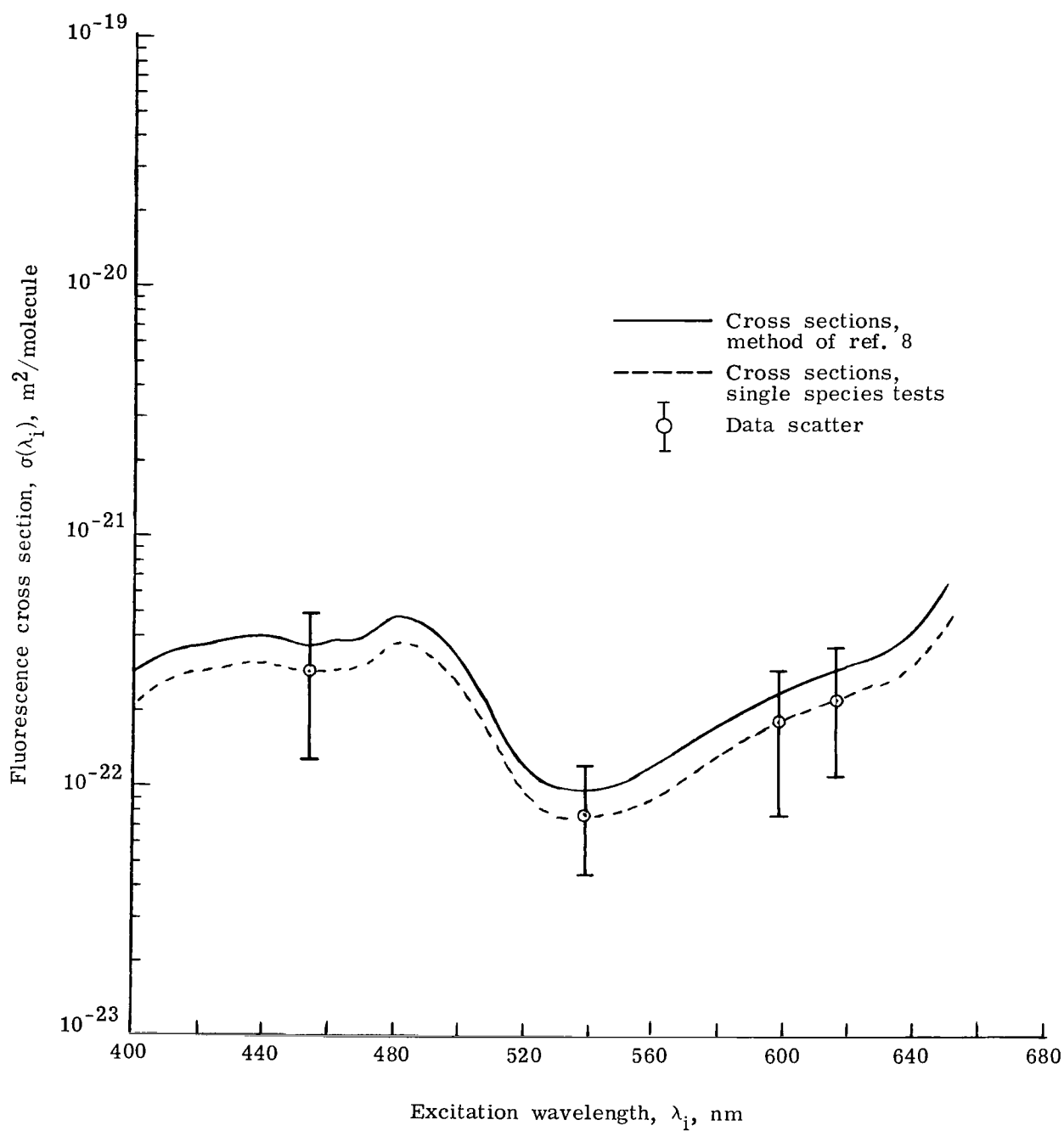
Figure 22.- Continued.

(d) Red algae, Va-70.

Figure 22.- Continued.

(e) Diatom algae, Va-72.

Figure 22.- Continued.

(f) Green algae, Va-74.

Figure 22.- Concluded.

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15. Supplementary Notes

16. Abstract

Phytoplankton were grown in the laboratory for the purpose of testing a remote fluorosensor developed at the NASA Langley Research Center. The fluorosensor uses a unique four-wavelength dye laser system to excite phytoplankton bearing chlorophyll a and to measure the chlorophyll a fluorescence generated by this excitation. Six different species were tested, one at a time, and each was grown two to four times.

The results shown in data from these tests are (1) fluorescence measured by the fluorosensor provides good quantitative measurement of chlorophyll a concentrations for all species tested while the cultures were in log phase growth (except for tests with the blue laser on Va-9 and Va-70); (2) fluorescence-cross-section ratios obtained in the single species tank tests support the hypothesis that the shape of the fluorescence-cross-section curve remains constant with the species (differences in fluorescence-cross-section ratios are a basis for determining composition of phytoplankton according to color group when a multiwavelength source of excitation is used); and (3) linear relationships exist between extracted chlorophyll a concentration and fluorescence measured by the remote fluorosensor during the log phase growth of phytoplankton cultures tested.

17. Key Words (Suggested by Author(s))	18. Distribution Statement
Fluorosensor Chlorophyll a Phytoplankton Remote sensors Cross sections Oceanography Fluorescence Biology	Unclassified – Unlimited Subject Category 43

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