# Daily patterns of fluorescence in vivo in the central equatorial Pacific

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ABSTRACT

A daily cycle of fluorescence *in vivo* was strikingly apparent in surface waters of the central equatorial Pacific between latitudes 4N and 10S, but not in waters to the north or south of this zone. These changes in fluorescence did not represent changes in chlorophyll-*a* concentration, but rather a photoinhibition of fluorescence by ambient light. Higher nutrient and chlorophyll-*a* concentrations were found in the region where cycling occurred.

The latitudes 4N to 10S are the approximate boundaries of the South Equatorial Current, a distinct phytogeographical region. The daily cycle in fluorescence seems to be a species specific response. When it occurs, such cycling presents a major obstacle to surface mapping of chlorophyll distributions.

## 1. Introduction

During extended periods on Cruise 79-G-6 (Fig. 1) of the R/V *Gyre,* we made continuous underway measurements of fluorescence *in vivo,* of temperature, and of nutrients in surface waters of the Pacific in an attempt to map chlorophyll distributions. This cruise was principally for geochemical and geophysical purposes; fluorescence measurements were actually taken to be correlated with chemical properties in the surface water, but proved to be of considerable interest on their own.

Our cruise track traversed the current systems of the north and south equatorial Pacific. Approximate boundaries of these currents are shown in Figure 1. The South Equatorial Current transports a tongue of nutrient-rich seawater from the upwelling area off South America (Desrosieres, 1969). Reid (1962) mapped the phosphate concentration of the Pacific Ocean and found higher phosphate concentrations along the equator with values decreasing to the west. Within the boundaries of the South Equatorial Current, the Equatorial Divergence is an area where nutrient-rich subsurface water upwells. Such regions are characterized by high primary productivity.

Site A in the South Equatorial Current (Fig. 1) was surveyed during the period

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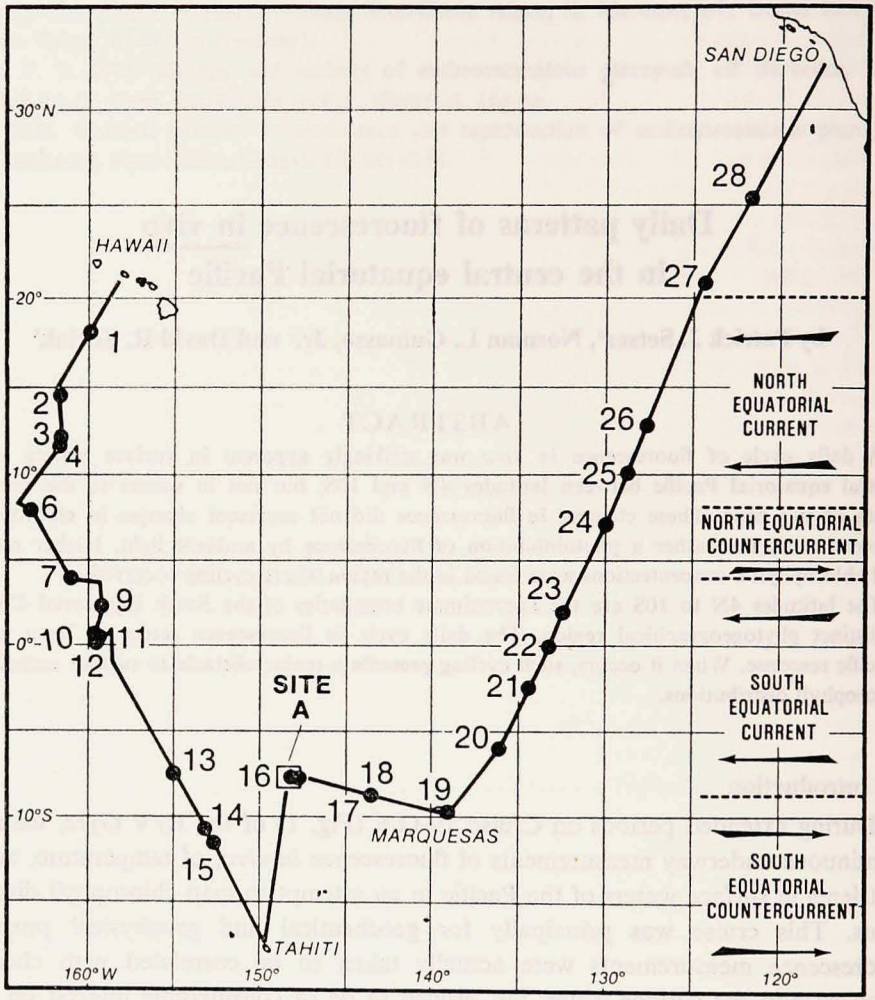



Figure 1. Cruise track and stations of Cruise 79-G-6. Approximate current boundaries are shown at right. "Site A" surrounds Station 16, Leg 1 was from Hawaii to Tahiti, Leg 2 was from Tahiti to Marquesas Islands, Leg 3 to San Diego.

from August 21 to August 26; August 24 and a portion of August 25 were spent on Station 16. Over the period of the "Site A" survey, the continuous *in vivo* fluorescence followed a daily cycle that was not directly correlated with changes in chlorophyll-*a* concentration.

Continuous fluorometry offers the best available technique for quantitative mapping of phytoplankton distributions in the horizontal plane, but there are many potential sources of error. The technique is based on the shaky assumption that a constant ratio exists between *in vivo* fluorescence and chlorophyll concentrations.

Flemer (1969) pointed out several sources of variation in this ratio: changes in species composition, physiological state, the amount of detrital chlorophyll, and temperature.

Kiefer (1973a) found *in vivo* fluorescence could vary greatly, while chlorophyll concentrations remained relatively constant. He reported photoinhibition of the fluorescence by phytoplankton in the surface waters of the Gulf of California when these phytoplankton were exposed to solar irradiance exceeding 0.15 langley $min^{-1}$. Kiefer (1973b) also reported large variations in the cellular fluorescence of marine centric diatoms in response to light and nutrient stress. *In vivo* fluorescence of chlorophyll-*a* declined when exposed to high light intensities with the chloroplast contracting and migrating to valvar ends of the cells. Both the contraction and aggregation of the chloroplast caused a decrease in fluorescence *in vivo*. Loftus and Seliger (1975) observed dramatic variations in fluorescence between day and night in Chesapeake Bay, particularly when diatoms dominated the phytoplankton biomass.

These variations in cellular fluorescence have been related to structural and chemical changes in the chloroplast. Marra (1978) studied the photosynthetic response to a daily varying light regime in cultures of the marine diatom *Lauderia borealis*. He reported a decline in fluorescence *in vivo* per cell with increasing light intensity, but did not observe chloroplast migration in response to high light intensity. Heaney (1978) found photoinhibition of fluorescence *in vivo* at high values of irradiance in cultures of several fresh-water phytoplankton. Karabashev and Solovyev (1977) reported a diel variation in fluorescence *in vivo* along the equator in the Pacific Ocean at stations located between 97W and 155W and at several stations perpendicular to the coast in the Peruvian upwelling. *In vivo* fluorescence reached a maximum toward midnight and decreased toward noon, in some places by an order of magnitude. They found the largest oscillation amplitude at 5 m depth; the oscillation had almost disappeared at 100 m.

## 2. Methods

Continuous measurements of fluorescence were made using a Turner Designs Model 10 fluorometer and a flow-through cuvette. A blue fluorescent lamp (F4T5) was used to excite the sample between 400 and 500 nm. The excitation filter was blue (Corning CS 5-60) with maximum transmission at 430 nm. The red emission filter (Corning CS 2-64) was of the sharp cut type being opaque to light at wavelengths shorter than 645 nm. An infrared-sensitive photomultiplier (R446) was used as the detector. *In vivo* fluorescence varied over one order of magnitude. Fluorometer sensitivity scales of ×100, ×3.16 to ×100, ×31.6 were used, then all values were normalized to the ×100, ×3.16 scale.

Surface seawater was supplied to the laboratory either from the ship's sea-chest which samples from between 3-4 m depth, or by pumping from a "fish" towed

approximately 2 m off the starboard beam at depths varying between 0-3 m, but usually between 0.3 and 1 m. A Vanton Flexiliner pump inside a laboratory on the main deck drew water from the fish and delivered it to a laboratory on the deck above. The main deck of the *Gyre* stands 1 m above the waterline, but the suction lift is reduced somewhat by the dynamic force on the fish dragging through the water with the hose intake in its nose. On Leg 1 the hose from fish to pump was translucent nylon, thereafter it was nylon-lined, with opaque covering. Short sections of Tygon® tubing at the pump allowed inspection of the water as it flowed. We were able to observe a number of very tiny bubbles in the inlet stream, presumably due to exsolution of dissolved gases in the water as it was sucked aboard at less than 1 atm pressure. On the outlet side of the pump these bubbles were not apparent—a fact attributed to the increased pressure on discharge. An opaque hose carried the water at a flow rate of 6 *l*/min to the laboratory 3 m above the main deck. There it was split and sent to several instruments; only a small fraction —still carried by opaque hose—passed through the fluorometer at varying flow rates. Because the stream split near the instrument and because flow rate of the main stream was nearly constant, residence time of water in the hose was also nearly constant at ~100 sec. *In vivo* fluorescence in water obtained from the sea-chest was carefully checked and found to be the same as water obtained from the fish; residence time of this water in pipes and hose was also ~100 sec. Close inspection of the water stream leaving the fluorometer revealed no bubbles. However, the towed fish would occasionally break the water surface and send a packet of bubbles through the water stream. When this happened the effect on the fluorometer reading was dramatic and unmistakable: apparent fluorescence increased and became highly variable. These artifacts were deleted from the data set.

Discrete samples were collected periodically from the fluorometer effluent and at each station for chlorophyll-*a* and phaeopigment-*a* analysis. The analytical technique used to determine chlorophyll-*a* and phaeopigment concentration was adapted from the methods of Yentsch and Menzel (1963), Lorenzen (1966), Loftus and Carpenter (1971), Strickland and Parsons (1972), and Kiefer (1973c). Water samples of 500 ml to 1 *l* of water were passed through a 105 $\mu$m-mesh plankton net to remove zooplankton. The water was then filtered under vacuum onto Whatman GF/C glass fiber filters, with suction held to less than ⅓ atmosphere to prevent cell lysis and subsequent loss of chlorophyll. While some water still remained to be filtered, 1 ml of magnesium carbonate solution was added to the water to prevent the phytoplankton chlorophyll from becoming acidic and decomposing to phaeophytin pigments.

Since chlorophyll decomposes quickly in light, extractions and measurements were carried out in subdued light. The filter was placed in a culture tube and 10 ml of 90% aqueous acetone was added. The tube was stoppered and the filter disintegrated by vigorous shaking. The pigments were extracted by placing the tube in

a refrigerator for at least 20 hours. The extracts were then allowed to come to room temperature in the dark and transferred to a centrifuge tube and centrifuged for one minute. The fluorescence of the extract was then measured. In no case was fluorometer sensitivity greater than $\times 100 \times 10$ scale required.

Relative abundance of phaeopigment was evaluated in each sample by observing the loss in fluorescence upon addition of acid. Two drops of 1N HCl were added to the cuvette and the fluorescence remeasured within three minutes. The ratio of fluorescence before:fluorescence after acidification remained nearly constant at 1.7 to 1.9; variations in the proportion of phaeopigments did not seem to contribute to any of the observed changes in pigment concentrations.

The fluorometer was standardized against a known chlorophyll-*a* standard (spinach extract, Sigma Chemical Co.) as determined using the spectrophotometric equation given by Jeffrey and Humphrey (Humphrey, 1978). Coproporphyrin tetramethyl ester in HCl was used as a secondary standard. The calibration of the fluorometer did not change throughout the cruise. Background fluorescence of the seawater was not measured but has been reported to be small for waters of this type (Herbland, 1978).

Several additional measurements were made on the water stream. Temperature of the fluorometer effluent was monitored; *in situ* temperature was also continuously recorded using a Leeds & Northrup thermograph with a platinum resistance thermometer. Relative solar irradiance was followed with a Clairex Corporation photocell. Nutrient concentrations were determined photometrically using the Technicon Auto-Analyzer II system: silicate by the reduction of silico-molybdic acid to the blue silico-molybdous form (Brewer and Riley, 1966); phosphate by the formation of phosphomolybdenum blue complex (Murphy and Riley, 1962); nitrate by a diazo compound coupling with N-1-naphthylethylenediamine dihydrochloride to form a soluble dye; nitrate by reducing nitrate to nitrite by a copper-cadmium reduction column (Armstrong *et al.*, 1967).

Phytoplankton samples were collected for future identification, but buffer added to the formalin proved unstable and the samples were spoiled before identification.

## 3. Results

During the "Site A" survey, *in vivo* fluorescence of surface waters showed strong daily cycles with values at night up to four times higher than those obtained during the day. During this period, first daylight was at about 0500L; last light faded at about 1900L. Figure 2a shows a portion of that survey. To further illustrate this daily cycle we have plotted the fluorescence against hour of the day in Figure 2b and overlaid values from five days of the survey period. Fluorescence values differed somewhat from one night to the next, but daylight fluorescence values were relatively consistent over the whole period. *In vivo* fluorescence showed greater variance at night than during daylight.

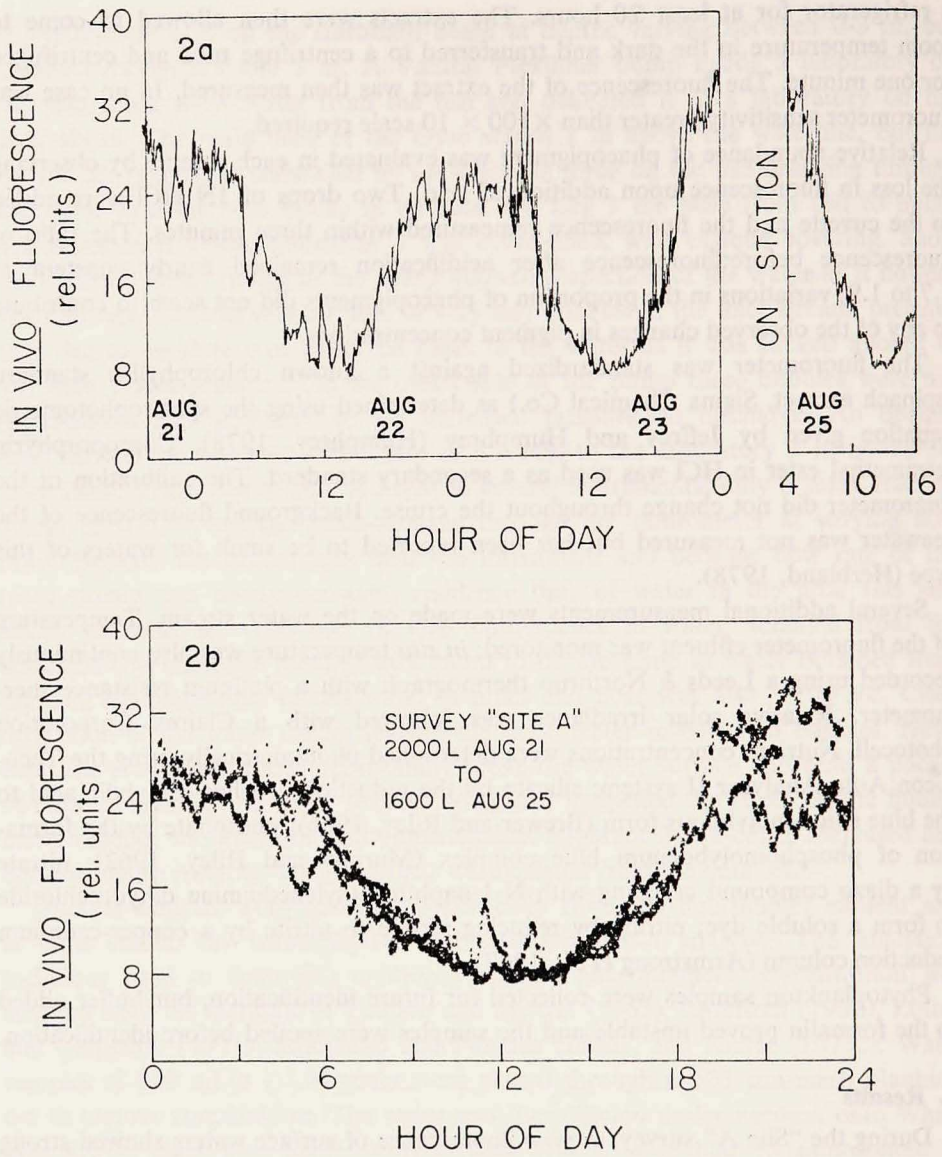



Figure 2. (a) Continuous *in vivo* fluorescence (relative units) in surface waters at survey "Site A" from Aug. 21-Aug. 25. A break in data collection occurs from midnight of Aug. 23 to 0400L on Aug. 25. Sensitivity scale: ×100 × 3.16. (b) Hourly surface *in vivo* fluorescence. Fluorescence values from Aug. 21 to Aug. 25 have been overlayed.

Our principal light-measuring instrument did not function during this cruise. The back-up photocell system provided only relative light intensity values and saturated at relatively low intensities, usually from about one hour after sunrise

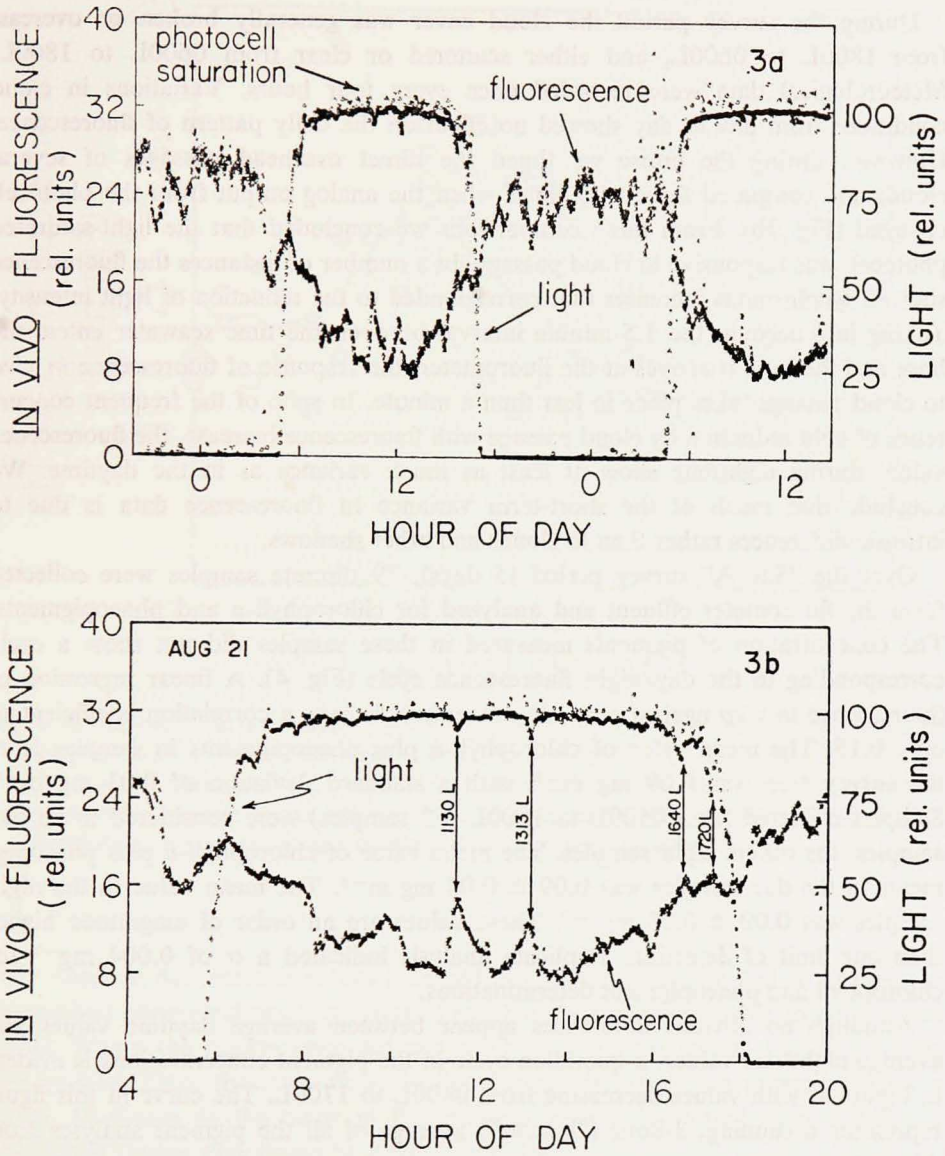



**Figure 3.** (a) Continuous surface *in vivo* fluorescence (relative units) and relative light intensity from 2000L, Aug. 21 to 1500L, Aug. 23. Sensitivity scale: ×100 × 3.16. (b) Enlargement of data from 0400L to 2000L, Aug. 21. Arrows indicate times where changes in light intensity and *in vivo* fluorescence could be correlated.

until one hour before sunset. Figure 3 shows representative light intensity data as obtained with the photocell. The relation between continuous *in vivo* fluorescence and ambient light is apparent in Figure 3a.

During the survey period the cloud cover was generally broken or overcast from 1800L to 0600L, and either scattered or clear from 0600L to 1800L. Meteorological data were recorded once every four hours. Variations in cloud conditions from day to day showed no effect on the daily pattern of fluorescence. However, during the cruise we timed the direct overhead passages of several clouds and compared these with times when the analog output from the photocell changed (Fig. 3b). From these comparisons we concluded that the light-saturated photocell was responsive to cloud passage. In a number of instances the fluorescence showed synchronous increases that corresponded to the reduction of light intensity. Taking into account the 1.5-minute interval between the time seawater enters the hose and the time it arrives at the fluorometer, the response of fluorescence *in vivo* to cloud passage takes place in less than a minute. In spite of the frequent concurrence of light reduction by cloud passage with fluorescence increase, the fluorescence values during nighttime show at least as much variance as in the daytime. We conclude that much of the short-term variance in fluorescence data is due to intrinsic differences rather than to clouds and other shadows.

Over the "Site A" survey period (5 days), 79 discrete samples were collected from the fluorometer effluent and analyzed for chlorophyll-*a* and phaeopigments. The concentration of pigments measured in these samples did not show a cycle corresponding to the day/night fluorescence cycle (Fig. 4). A linear regression of fluorescence *in vivo* against pigment concentration gave a correlation coefficient of only 0.15. The mean value of chlorophyll-*a* plus phaeopigments in samples from the survey area was 0.09 mg $m^{-3}$, with a standard deviation of 0.03 mg $m^{-3}$. Samples collected from 0500L to 1900L (52 samples) were considered to be day samples, the others night samples. The mean value of chlorophyll-*a* plus phaeopigments in the day samples was 0.09 $\pm$ 0.04 mg $m^{-3}$. The mean value in the night samples was 0.09 $\pm$ 0.03 mg $^{-3}$. These values are an order of magnitude higher than our limit of detection. Replicate analysis indicated a $\sigma$ of 0.004 $mg^{-3}$ for chlorophyll and phaeopigment determinations.

Although no obvious differences appear between average daytime values and average nighttime values, a quotidian cycle in the pigment concentrations is evident in Figure 4, with values decreasing from 0800L to 1700L. The curve in this figure represents a running, 3-hour ("boxcar") average of all the pigment analyses from this area.

No distinct quotidian cycles in the *in vivo* fluorescence or in chlorophyll concentration were observed outside the zone from 4N to 10S. Continuous *in vivo* fluorescence profiles from other regions (Fig. 5) illustrate the absence of such cycles. Continuous runs of over 24 hours were rare on this cruise due to stops for hydrographic stations. The profile in Figure 5c was obtained over a 33-hour period at the edge of the region where daily cycling occurred.

When the quotidian cycles of fluorescence *in vivo* did occur, they had a wider

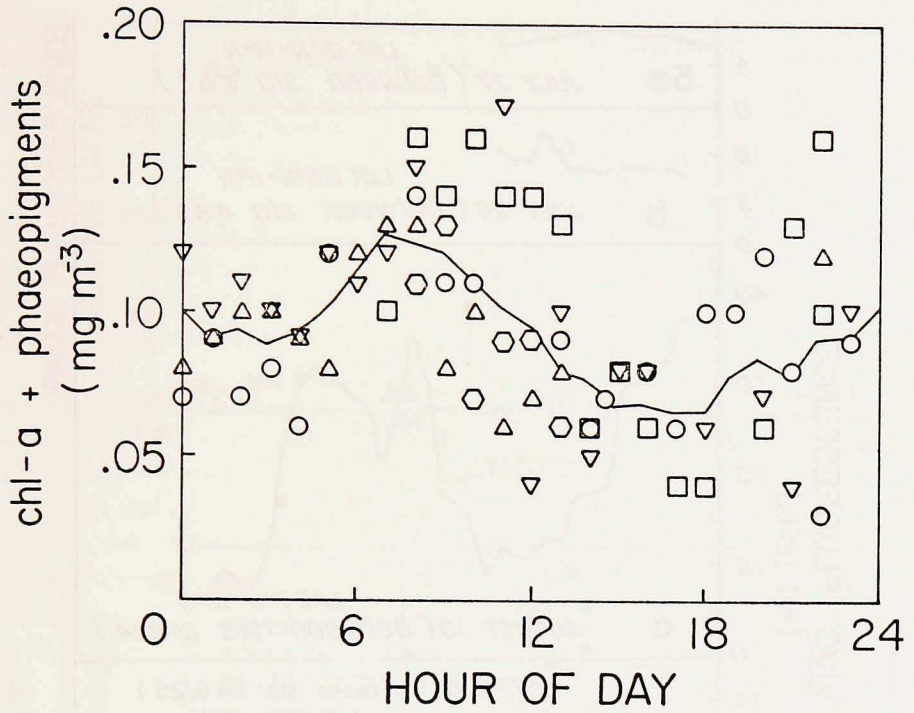



Figure 4. Chlorophyll-*a* plus phaeopigments concentration by hour of day. Seventy-nine discrete surface *in vivo* samples were analyzed from Aug. 21 (△), Aug. 22 (○), Aug. 23 (□), Aug. 25 (hexagon) and Aug. 26 (▽). Replicate samples had a standard deviation ($1\sigma$) equal to the size of the symbols. Solid line represents the generalized daily pigment concentration, derived by taking a running 3-hour average of all samples.

daily range in fluorescence values than did any variations attributed to changes in chlorophyll concentrations (e.g., from 8 relative units at noon to the low-thirties at night). Where the fluorescence did not exhibit these daily cycles the daily range was smaller. Thus, the daily range in fluorescence indicates the presence of daily cycles. In Figure 6a the range of fluorescence *in vivo* is plotted against latitude. The largest ranges were found between 10S and 4N.

Figure 6b shows surface concentrations of nutrients and of *in vitro* chlorophyll-*a* plus phaeopigment concentrations from the hydrographic stations along our cruise track. Highest values again were found between 4N and 10S. North of 4N the nitrate values were below detectable levels (with the exception of Station 4). No daily cycle in fluorescence was observed where surface water nitrate concentrations were zero. The relatively low value of pigments found at Station 13 (7°40′S latitude) may be due to an invasion of waters from a region of lower nutrients;

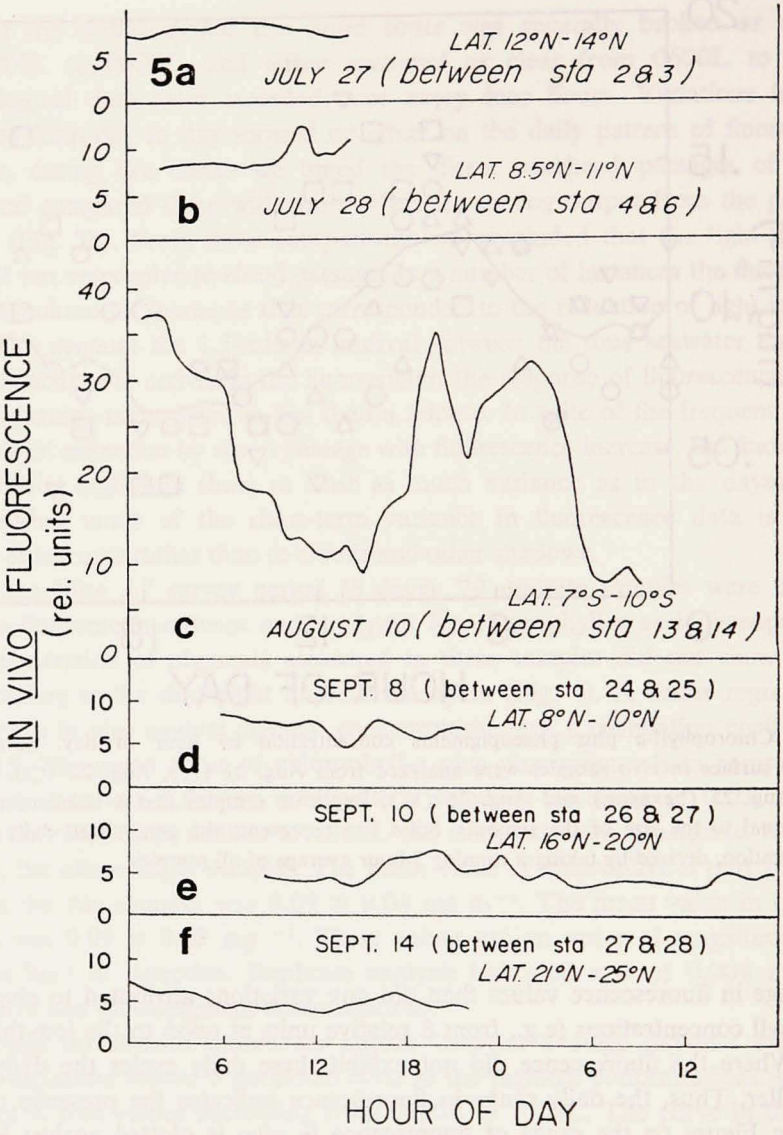



**Figure 5.** Representative profiles of continuous *in vivo* fluorescence (relative units) in surface waters from Leg 1 (a,b,c) and Leg 3 (d,e,f). Sensitivity scale: ×100 × 3.16.

this station was at the edge of the higher nutrient area, and two separate hydrographic casts taken there showed surface nitrate concentrations of 1.2 $\mu$M and 2.6 $\mu$M, respectively. The low chlorophyll-*a* value at Station 13 was obtained from the cast with the low nitrate concentration.

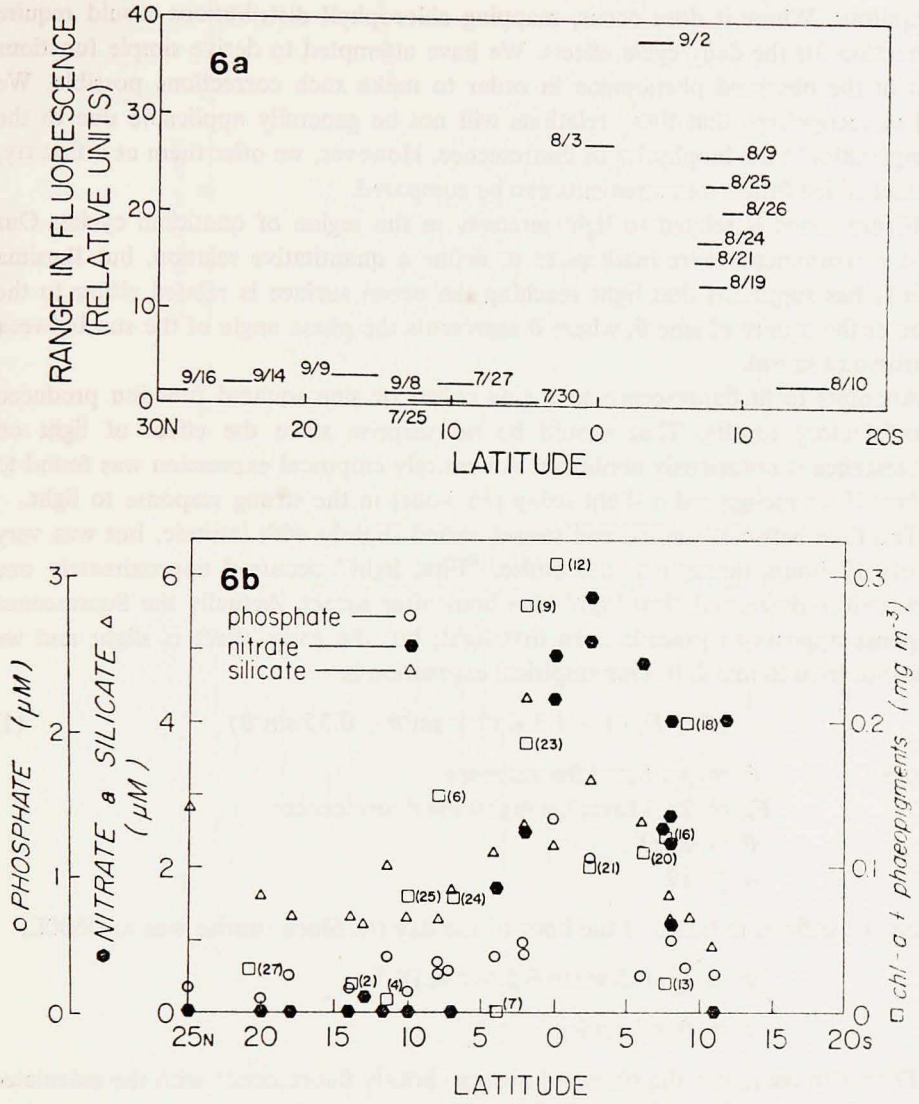



Figure 6. (a) Range of surface *in vivo* fluorescence by latitude. Range in fluorescence is difference between day and night values. Month and day are shown. (b) Surface nutrients and pigment concentrations by latitude. Values are from stations along the cruise track. Station number is indicated next to pigment value.

## 4. Discussion

The effect of light on fluorescence, as shown in Figures 2 and 3, has been observed before. Kiefer (1973a, b) and Loftus and Seliger (1975) have offered excellent descriptions of the effect. Our data clearly indicate that this effect is not

ubiquitous. Where it does occur, mapping chlorophyll distributions would require correction for the daily cycle effects. We have attempted to derive simple functions that fit the observed phenomena in order to make such corrections possible. We did so recognizing that these relations will not be generally applicable due to the complexities in the biophysics of fluorescence. However, we offer them as a first try, against which future measurements can be compared.

Fluorescence is related to light intensity in the region of quotidian cycles. Our light measurements were inadequate to define a quantitative relation, but Ikusima (1967) has suggested that light reaching the ocean surface is related either to the cube or the square of sine $\theta$, where $\theta$ represents the phase angle of the sun between sunrise and sunset.

Attempts to fit fluorescence to a sine cubed or sine squared function produced unsatisfactory results. That should be no surprise since the effect of light on fluorescence is notoriously nonlinear. An entirely empirical expression was found to fit best if we recognized a slight delay (½ hour) in the strong response to light.

The time between sunrise and sunset varied slightly with latitude, but was very nearly 12 hours throughout the cruise. "First light" occurred approximately one hour before dawn and "last light" one hour after sunset. Actually the fluorescence response appears to precede even first light, but the early effect is slight and we have not tried to match it. Our empirical expression is

$$F = F_n\,(1 - 1.3\,\sin\theta + \sin^2\theta - 0.35\,\sin^3\theta) \qquad (1)$$

where
$F$ = predicted fluorescence
$F_n$ = 25.4 (average nighttime fluorescence)
$\theta = \pi\tau/\lambda$
$\lambda = 12$

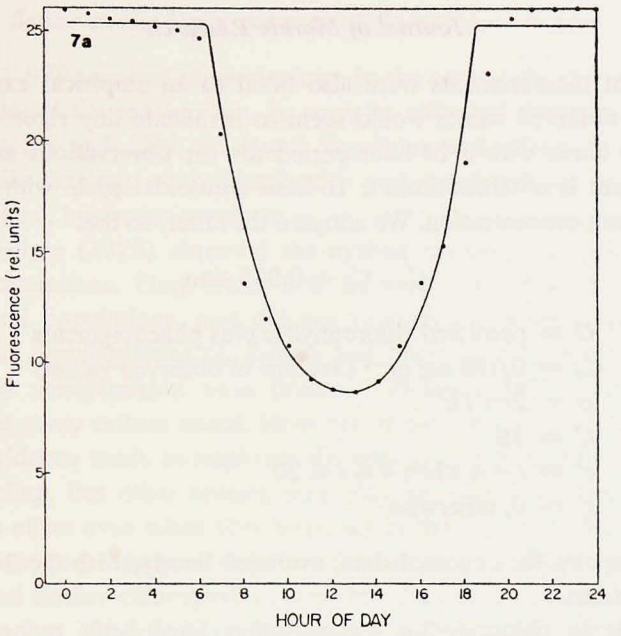
$\tau$ may be defined in terms of the hour of the day ($t$). Since sunrise was at 0600L:

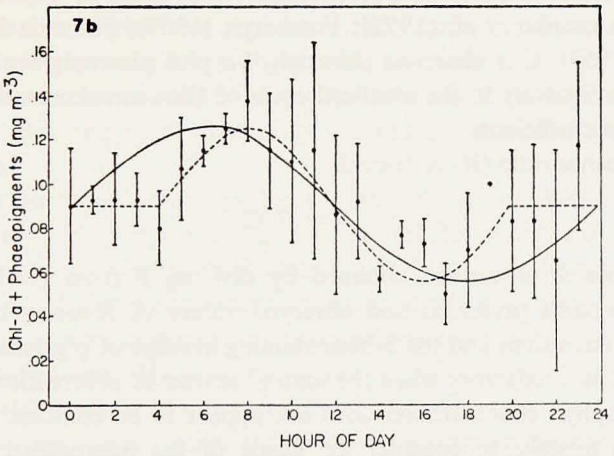
$\tau = t - 6.5$ when $6.5 \leqslant t \leqslant 18.5$

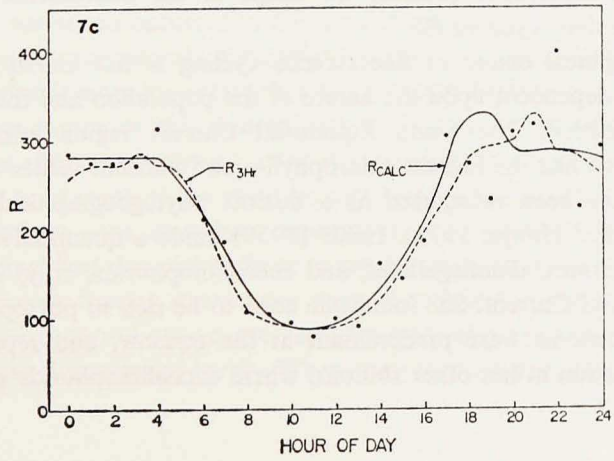
$\tau = 0$, otherwise

Figure 7a compares the observed average hourly fluorescence with the calculated value. Obviously closer fits could be obtained with other expressions, but these would probably require additional arbitrary constants. A more exact fit does not seem to be required, considering the nature of the data.

Figure 7. Hourly averages of collected observations in Survey Site A (points) plotted against time of day, compared with derived functions (lines). (a) *In vivo* fluorescence; curve represents the adopted empirical expression (Eq. 1). (b) Pigment concentrations; error bars represent $1\sigma$; curves show two functions that might represent the observed cycles. (c) $R$ values; curve ($R_{calc}$) represents the ratio obtained by dividing Eq. (1) by Eq. (2). $R_{3Hr}$ represents the ratio of hourly average fluorescence divided by the 3-hour running average of pigment concentration.


7a
Fluorescence (rel units)
HOUR OF DAY


7b
Chl-a + Phaeopigments (mg m-3)
HOUR OF DAY


7c
R
R3Hr
RCALC
HOUR OF DAY

Our pigment measurements were also fitted to an empirical expression. Again the scatter in observed values would seem to invalidate any rigorous formulation. A simple sine curve with a 24-hour period fits the observations adequately; even better agreement is obtained from a 16-hour sinusoidal cycle with 8 hours of essentially constant concentration. We adopted the latter, so that

$$C = C_n + 0.035 \sin\alpha \tag{2}$$

where
$C$ = predicted chlorophyll-*a* plus phaeopigments
$C_n$ = 0.090 mg m$^{-3}$ (average of observed values)
$\alpha = 2\pi\tau'/\lambda'$
$\lambda' = 16$
$\tau' = t - 4$ when $4 \leqslant t \leqslant 20$
$\tau' = 0$, otherwise

Figure 7b compares the collected data, averaged hourly, with the 24-hour and 16-hour sine functions.

Daily cycles in chlorophyll-*a* concentration have been reported by several workers: Glooschenko *et al.* (1972); Forsbergh (1969); Shimada (1958); Yentsch and Ryther (1957). Our observed chlorophyll-*a* plus phaeopigment concentrations correspond qualitatively to the idealized cycle of Glooschenko *et al.*, but do not fit their correction coefficients.

The fluorescence ratio ($R$) is defined:

$$R = F/C \tag{3}$$

Predicted values of $R$ can be obtained by dividing $F$ from (1) by $C$ from (2). Figure 7c compares predicted and observed values of $R$ using both the hourly averaged pigment values and the 3-hour running average of pigment concentrations. The agreement is satisfactory when the natural scatter of observations is considered.

Since chlorophyll concentration does not appear to be constant during the day, it would be a mistake to consider the shape of the fluorescence daily cycle as identical to the daily cycle in $R$.

The geographical extent of fluorescence cycling is not known, but the effect appears to be dependent upon the nature of the population and therefore its scope might be predicted. The South Equatorial Current region where fluorescence cycling occurred had the highest chlorophyll-*a* and nutrient values observed on the cruise. This has been recognized as a distinct phytogeographical zone (Semina, 1974; Okada and Honjo, 1973). Hasle (1959) made a quantitative study of phytoplankton (diatoms, dinoflagellates, and coccolithophorids, only) in waters of the South Equatorial Current. She found the zone to be rich in phytoplankton. In surface waters, diatoms were predominant at the equator, and represented a very substantial fraction at her other stations, where coccolithophorids predominated.

Fryxell *et al.* (1979) studied phytoplankton in the central Pacific to the north of the South Equatorial Current system. In samples collected during spring and fall seasons between 5N and 18N she found flagellates and others to dominate the populations, while diatoms, coccolithophorids and dinoflagellates were present in approximately equal, but lesser, numbers.

Loftus and Seliger (1975) observed the cycling response in some diatom and dinoflagellate populations. They found it to be most distinct when diatoms dominated the natural populations, and did not observe it, "when populations were dominated by the dinoflagellates *G. nelsoni* and *Prorocentrum minimum,* or when only unidentified nanoplankton were present." Heaney (1978) found the effect present in almost every culture tested. However, it did not occur in the blue-greens.

The above evidence tends to implicate diatoms as the principal contributors in fluorescence cycling, but other species may play an important role. Or, diatoms might cause this effect even when they were not in the majority. When Loftus and Seliger (1975) reported enhanced nighttime fluorescence in Chesapeake Bay, their three samples had similar chlorophyll content but the diatom concentrations ranged from $2 \times 10^6$ to $8 \times 10^6$ cells/liter. Since the upper value can represent no more than 100% of the phytoplankton population, and since the authors reported no great change in the nature of the population, we may assume that the lesser abundance of diatoms represented no more than 25% of the population—certainly not the majority. Still, this latter assemblage showed 3× enhancement in the nighttime fluorescence.

Effect of light on fluorescence has been related to the nutritional state of the population. Blasco (1973) reported that the ratio of fluorescence *in vivo* to chlorophyll-*a* in a diatom batch-culture increased when the cells became deficient in nitrate or phosphate. Using a continuous culture of *Cyclotella nana,* Kiefer (1973b) demonstrated that *in vivo* chlorophyll-*a* fluoresced more strongly in nitrogen-starved cells than in enriched ones. He also found that photoinhibition of cellular fluorescence increased with the state of nitrogen deficiency.

Our data show just the opposite; the greatest increase in fluorescence and the largest day-night variations occurred in the zone of greatest nutrient concentrations (Fig. 6). This does not necessarily contradict the earlier finding. Rather it suggests that species selection is more important than the nutritional state.

Since the exact source of this fluorescence cycling is not established and the distribution of the effect is largely unknown, it is essential to any chlorophyll survey that the presence or absence of the effect be ascertained. Loftus and Seliger (1975) suggested a technique—the stop-flow experiment. A population (dominated by diatoms) which displayed day-night effects showed increasing levels of fluorescence when the continuously flowing stream was stopped and held in the fluorometer for ~30 min. The rise occurred over a protracted period, and approached or slightly exceeded nighttime fluorescence levels. When they stopped the flow of a sample

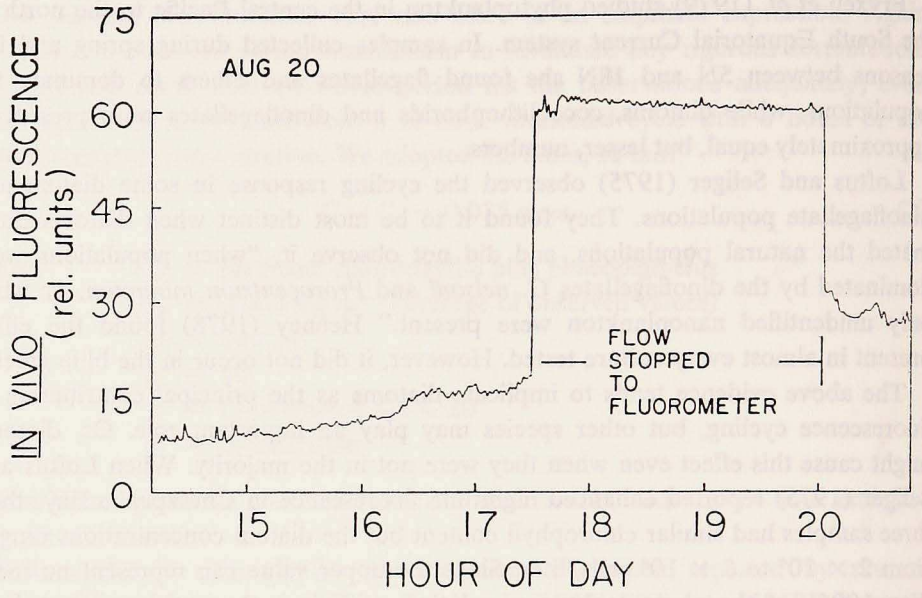



**Figure 8.** Response of *in vivo* fluorescence (relative units) from sample in cuvette during flow stoppage. Flow of surface water to the fluorometer was secured from 1730L to 2000L. During this period the cuvette remained filled with seawater.

not exhibiting a daily cycle, no increase in the fluorescence occurred; this type of sample was dominated by dinoflagellates and unidentified nanoplankton.

We performed such an experiment inadvertently on August 20, when flow from the fish to the fluorometer stopped from 1730L to 2000L. During this period the cuvette remained filled with surface seawater, and the algal cells present were exposed to the blue fluorescent lamp. This excites at wavelengths between 400 and 500 nm. The rotating shutter on the Turner Designs instrument interrupts the emitted light, not the excitation light, and thus offers no protection. Roy and Legendre (1979) give the lamp intensity as 0.025 ly $min^{-1}$, a level well below the photoinhibition threshold of 0.15 ly $min^{-1}$ reported by Kiefer (1973a). However, considerable caution is required when the intensity of a blue light is compared with the intensity of natural sunlight.

The effect we observed (Fig. 8) was even more dramatic than that found by Loftus and Seliger (1975). A four-fold increase occurred in fluorescence promptly after the flow stopped. The fluorescence remained at this elevated level (with only a slight decrease) until the flow to the fluorometer was resumed. When flow was resumed, the measured fluorescence dropped to the normal nighttime level (30 relative units).

To check the effect of the light source in warming the cuvette, a thermocouple was inserted into the flow cell and the temperature in the cell monitored after flow

stopped. In this test, and also during the cruise observations, water temperature was very nearly the same as laboratory temperature. When flow stopped, temperature in the fluorometer cell rose 1.0°C over a period of 5 min. Findings by other investigators (e.g., Lorenzen, 1966) lead us to believe that such temperature changes do not cause the observed changes in fluorescence. We have performed similar stop-flow experiments in the Gulf of Mexico where daily cycling did not occur. There we found no increase in fluorescence with flow stoppage.

These experiments indicate that stop-flow experiments offer a promising test for the presence of fluorescence cycling. Yentsch and Yentsch (1979) have shown some of the value of fluorescence spectral signatures. We speculate that the use of the entire excitation-emission spectra would also provide the information necessary to recognize when this effect is present. Instruments now available are capable of measuring these entire spectra at very rapid rates (Warner *et al.*, 1976).

## 5. Conclusions

Ambient light levels dramatically affected the *in vivo* fluorescence levels in some areas, but not in others. Proper correction for this effect should be based on continuous measurements of incident light along with measurements of fluorescence *in vivo*. Such adjustment could then lead to better correlation between the continuous underway fluorescence observations and the *in situ* chlorophyll-*a* concentrations.

Fluorescence did not change with ambient light levels in most regions. Where it did, the effect seemed primarily due to photoinhibition. The region of daily cycling corresponds to the region of the South Equatorial Current, a distinct phytogeographical region. We attribute the presence or absence of daily cycles to the composition of the phytoplankton population. Probably the daily cycles occur where diatoms form a significant part of the population.

Although this ambient light effect may be merely a nuisance requiring correction before chlorophyll-*a* concentrations can be mapped, we prefer to believe that the presence of daily cycles can be used to provide significant information about the character of the phytoplankton population. Change in fluorescence under "stop-flow" conditions also may offer information as to the nature of the fluorescence. We must try to establish whether "stop-flow" enhancement is always correlated with daily cycling. Continuous use of the full spectrum of excitation and emission might further enhance the value of these underway measurements.

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