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THE SOLUBLE FLUORESCENCE IN THE OPEN SEA: DISTRIBUTION AND ECOLOGICAL SIGNIFICANCE IN THE EQUATORIAL ATLANTIC OCEAN

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Abstract: The red fluorescence of filtered sea water has been measured on 216 samples in the 0–150 m layer of the equatorial Atlantic Ocean.

Soluble fluorescence is maximum where chlorophyll *a* and *in vivo* fluorescence are maximum, but the percentage of soluble fluorescence, (soluble fluorescence/*in vivo* fluorescence) × 100, is minimum at these levels; in recently upwelled waters of the equatorial divergence, the percentage of soluble fluorescence is equal to 10 in the 0–20 m layer and regularly increases to 60 or more at 100–150 m; in the nitrate depleted mixed layer of a convergence it averages 30, decreases to 15 in the thermocline maximum of chlorophyll *a*, and again reaches 60 in deep waters.

A significant positive correlation has been found between the percentage of soluble fluorescence and the amount of phaeophytin, and soluble fluorescence in the open sea is thought to be the result of the degradation and release of chloroplastic products by aged or grazed phytoplankton populations. Low values (<20) of the percentage soluble fluorescence indicate the presence of healthy phytoplankton cells, whereas high values (>30) are evidence of unfavourable growth conditions (*e.g.*, limiting nutrients or darkness) or high grazing pressure.

The simultaneous measurement of *in vivo* fluorescence and soluble fluorescence is a method of obtaining valuable information rapidly on the physiological state of the phytoplankton population in the water column.

INTRODUCTION

The measurement of *in vivo* fluorescence (Lorenzen, 1966) allows a rapid and easy estimate of phytoplankton abundance even in the most oligotrophic waters of the tropical ocean. Unfortunately, it is a total measurement which includes different fluorescent compounds and the conversion factor for chlorophyll *a* depends upon the species (Strickland, 1968; Blasco, 1973; Kiefer, 1973a), the physiological state of the cells (Kiefer, 1973b; Estrada, 1974); furthermore, it undergoes day–night variations (Blasco, 1973; Estrada, 1974).

Tunzi, Chu & Bain (1974) have pointed out that in some natural conditions (sloughs, lakes) the fluorescence of the filtered water amounted to 50–90% of the total sample fluorescence. In the water analysed by them, the soluble fluorescent compounds may have had a diverse origin (terrestrial detritus, phytoplankton, and phytobenthic products), but in the open sea, far from the shore, the soluble fluorescence (SF) probably has a single origin, namely the degradation or excretion of *in situ* phytoplankton. It has, therefore, an ecological significance in reflecting the physiological

state of the phytoplankton cells. The red SF has a different origin from the blue SF emitted when sea water is irradiated with an ultraviolet light. This last response is thought to be due to the presence of nanogram quantities of largely unidentified organic compounds (Kalle, 1966).

Except for the work of Kalle (1959) in the North Sea, no data have been published on the distribution of the red SF in the open sea in spite of his promising results.

This paper compares the vertical distribution of the red SF (hereafter referred to as SF), *in vivo* fluorescence (F), chlorophyll *a*, and phaeophytin in two contrasting hydrological conditions of the equatorial Atlantic Ocean, namely, an upwelling situation and a typical tropical situation.

Material and Methods

Data were collected during CAP 7706 cruise of the *R.V. Capricorne* in the equatorial Atlantic Ocean at 4 °W (Fig. 1).

The sampling levels were chosen from temperature profiles, with a closer sampling programme in the thermocline or in superficial waters. Temperature was recorded *in situ* with a STDO probe system (Bisset Berman) coupled with a Hewlett Packard computer. The samples of sea water were collected in 1.7 l P.V.C. bottles on a Rosette sampler (General Oceanics) at 12 levels between 0 and 150 m.

Nitrate analysis was made on an autoanalyser (Technicon) as described by Strickland & Parsons (1972).

For chlorophyll *a* measurement, 170 ml of sea water were filtered through Whatman GFC glass fibre filters with a low suction pressure (<75 mm Hg); after grinding, extraction in 90% acetone, and centrifugation the chlorophyll *a* was determined according to Holm-Hansen *et al.* (1965) using a Turner fluorometer (Model 111). A blue fluorescent lamp (F4T5) was used to excite the sample; the primary filter (excitation) was blue (Corning CS 5–60) with maximum transmission at 430 nm; the red secondary filter (Corning CS 2–64) was of the sharp cut type, being opaque to light at wavelengths shorter than 645 nm and reaching its maximum transmission at 650 nm. The values were corrected for the interference of phaeophytin by acidification. For calibration pure chlorophyll *a* (Sigma) was used.

In vivo fluorescence was measured by replacing the standard door of the fluorometer by a more sensitive one (Ref. 110880) modified in order to introduce discrete samples into the apparatus. The soluble fluorescence was measured after gentle filtration of the sea water on a fibre glass filter (Gelman Type A) with the same apparatus. The measurement of SF requires the following precautions: 1) after each filtration, the filtration apparatus must be carefully rinsed with distilled water, particularly the fritted glass where organic material is trapped; 2) only one cell must be used and it must be positioned in the same manner, because on

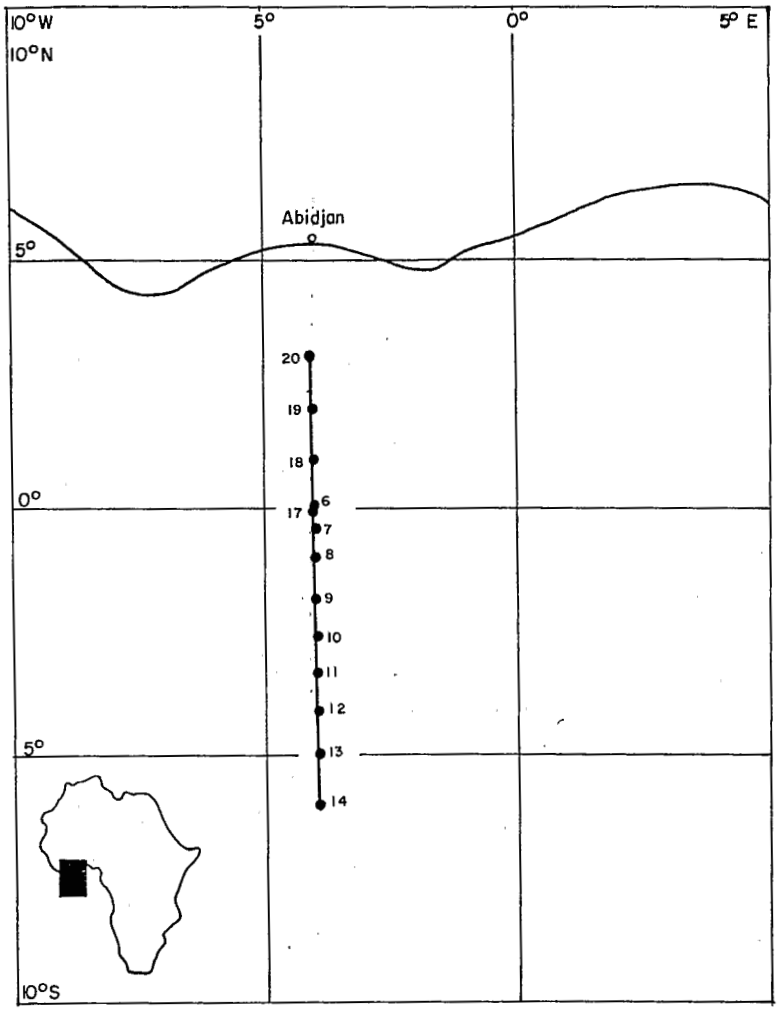

Fig. 1. Position of stations in the equatorial Atlantic Ocean where soluble fluorescence was measured.

30 × scale with the high sensitive door, the responses are variable from one cell to the other or from one position to the other; and 3) the zero, read on distilled water, must be frequently controlled, at least every five samples.

RESULTS

HYDROLOGICAL SITUATIONS

Two different hydrological situations were found. Between the equator and 6°S, an upwelling situation characterized by a decrease in temperature in superficial

waters (less than 22 °C at the surface between 0°30′S and 2°S, Fig. 2) was present. Here the thermocline had a slight gradient; the nitrate exceeded 2.0 μg-at. NO_3-N/l in surface waters and was not detectable only at 6°S (< 0.1 μg-at. NO_3-N/l) in the 0–20 m layer, where the northern boundary of the tropical convergence was crossed

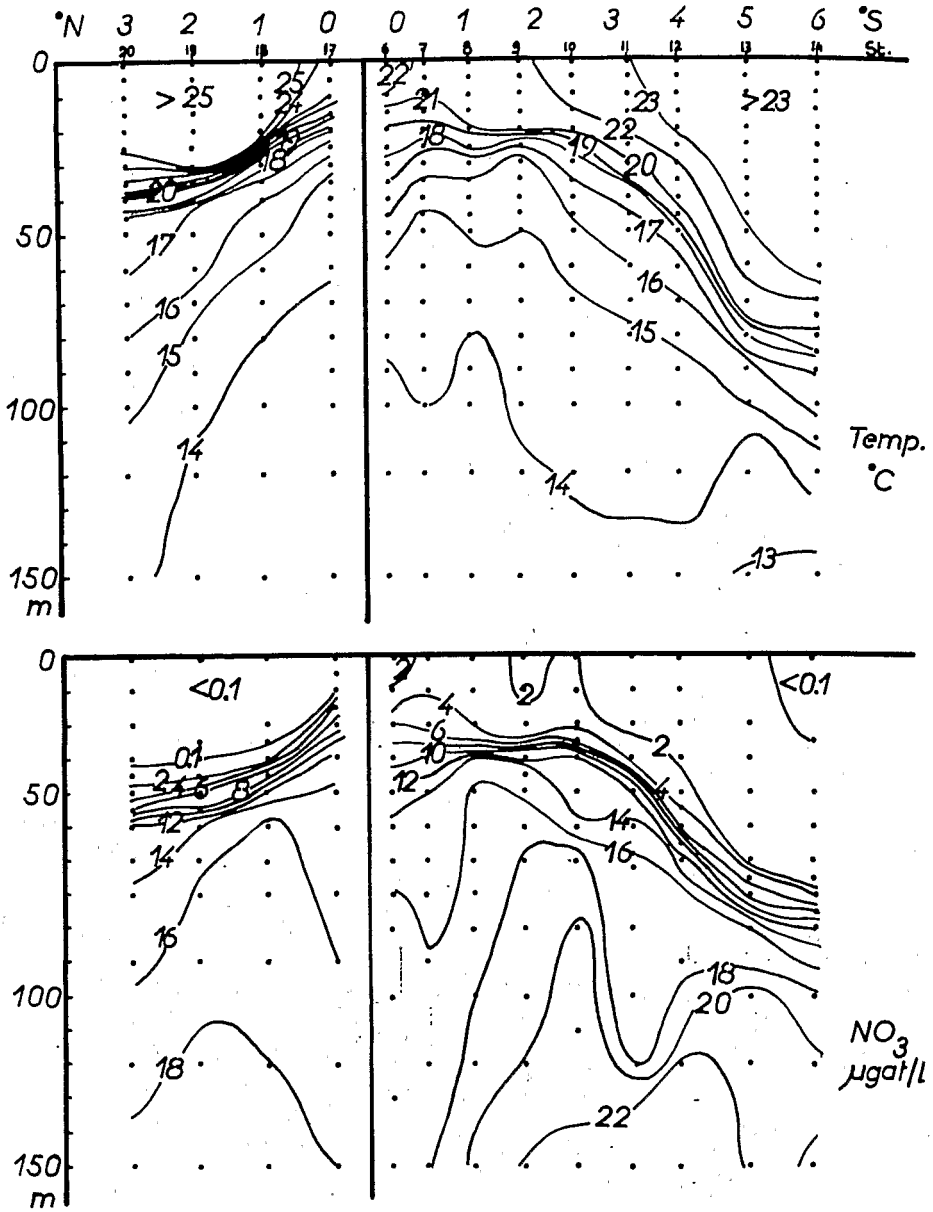

Fig. 2. North–south section showing the distribution of temperature and nitrate: there were 4.5 days between Station 6 and Station 17.

(Lemasson & Rebert, 1973). These results agree with those of Voituriez & Herbland (1977) in the same area at the same season. They pointed out an important superficial cooling during the boreal summer with a spectacular enrichment of nitrate in surface waters (>5 μg-at. NO_3-N/l). Between the equator and 3°N, there was a typical tropical situation as defined by Herbland & Voituriez (1977), characterized by a warm (>25 °C) nitrate-depleted layer and a sharp thermocline (Fig. 2). It is the southern part of the north equatorial convergence where the thermocline deepens and the density gradient increases (Donguy & Prive, 1964; Hisard, 1975).

CHLOROPHYLL *a*, *IN VIVO* FLUORESCENCE, AND SOLUBLE FLUORESCENCE

For each hydrological situation the distribution of chlorophyll *a* is different. In the upwelling, the maximum (0.30–0.40 μg chl *a*/l) is in the superficial layer (0–40 m) with sometimes a weak thermoclinal maximum (Fig. 3). In the typical tropical situation the maximum of chlorophyll *a* (0.20–0.30 μg chl *a*/l) is narrow and located in the nitracline with very low values in the nitrate-depleted layer (<0.10 μg chl *a*/l). The *in vivo* F has the same distribution (Fig. 3), *i.e.*, with a thick and superficial maximum in the upwelling situation and a narrow thermoclinal one in the tropical situation.

The SF has the same pattern of distribution but the absolute maximum lies between 20 and 40 m in the upwelling area. The values there are slightly greater (40–50 units on 30 × scale) than the values in the tropial situation (30–40 units on 30 × scale). In contrast, the percentage SF has an inverse distribution. In the upwelling situation it increases from the surface to the bottom (Fig. 3) while in surface waters it is low (=10), reaching 60 or more at 150 m; in the typical tropical situation is it relatively high in the mixed layer (=30), minimum (=15–20) at the level of chlorophyll *a* maximum and increases in deep waters (Fig. 3).

DISCUSSION

In the two opposite situations, the amount of SF is maximum where the chlorophyll *a* and *in vivo* F are maximum whereas the percentage SF is minimum at these levels (Fig. 4). It is well known that the maximum of chlorophyll or *in vivo* F corresponds to optimal growth conditions within the water column. We may therefore infer that the SF is derived from the *in situ* phytoplankton development since its value is maximum where the phytoplankton is most abundant, and that it is due to decomposed pigments since its percentage is low where the growth conditions are favourable and increases where the nutrients (mixed layer) or light (deep levels) are limiting.

Tunzi, Chu & Bain (1974) noted that during the growth of an algal culture under optimal conditions the observed increase in SF was slight in comparison to the total *in vivo* F of the unfiltered culture.

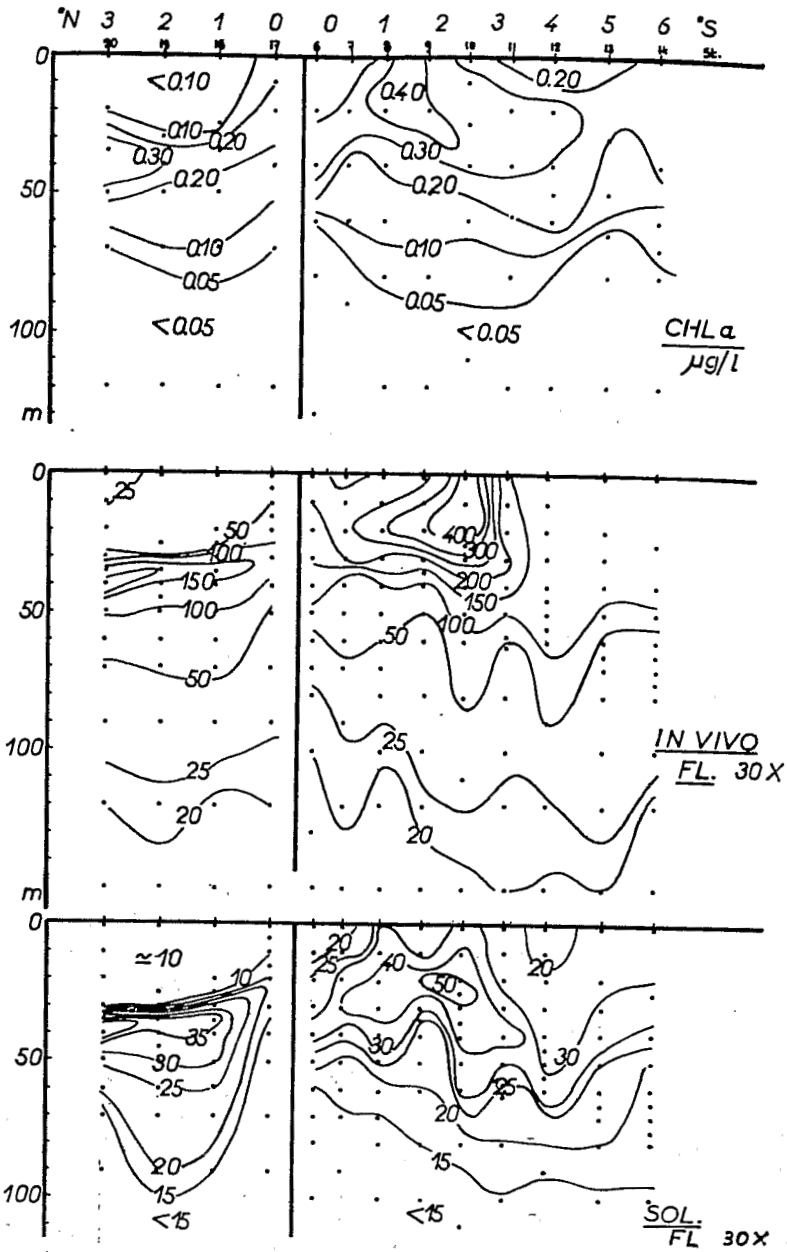

Fig. 3. North–south section showing the distribution of chlorophyll *a*, *in vivo* fluorescence, soluble fluorescence, and percentage soluble fluorescence.

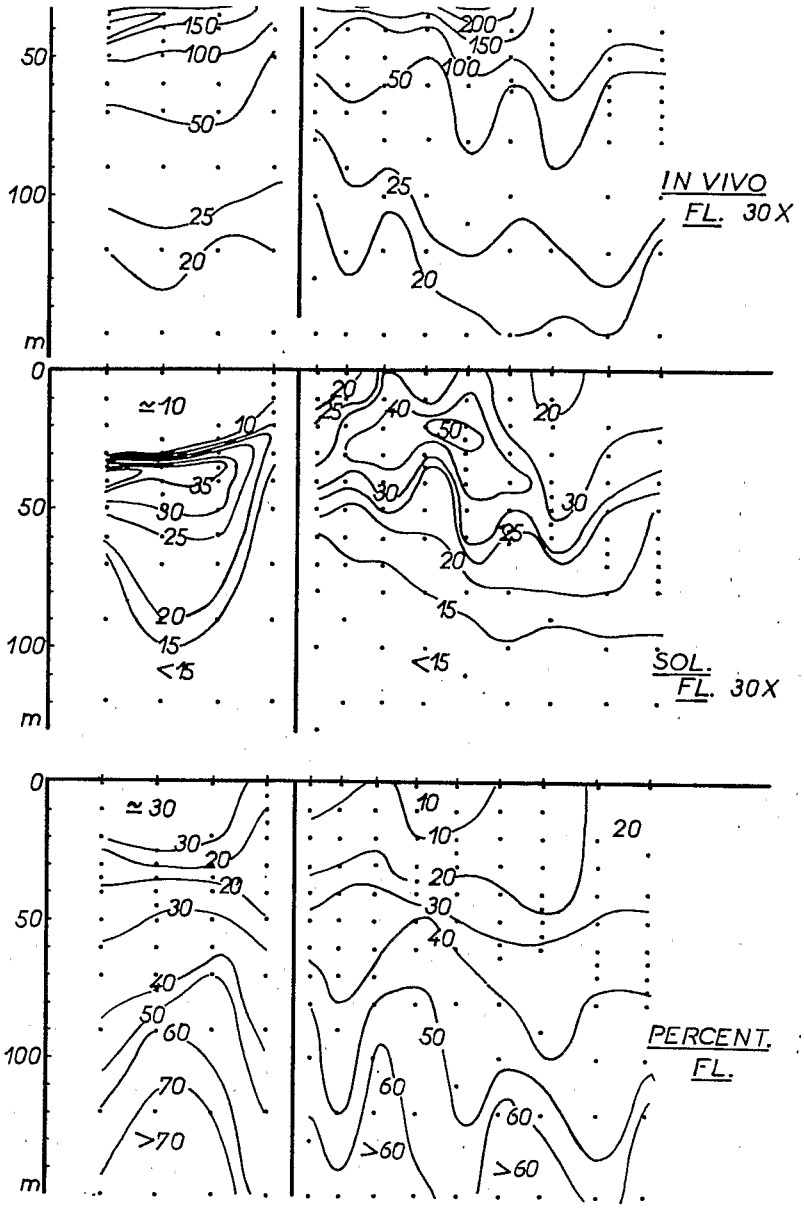

Fig. 3, continued. Station numbers and latitudes as on opposite page.

In order to show whether soluble chlorophyll *a* was present in our samples several filtrates were acidified in the same manner as the acetone extracts. No noticeable change could be found in the fluorometer reading when acid was added and the SF is, therefore, due to already decomposed products of chlorophyll.

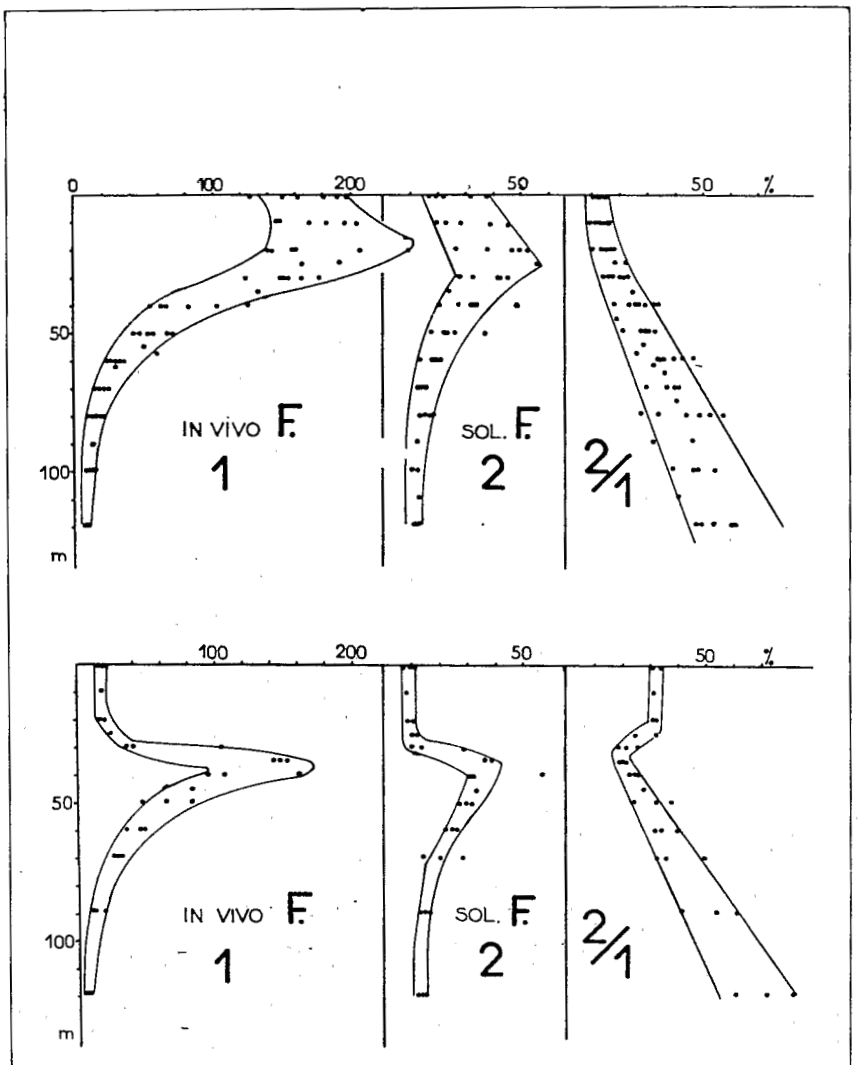

Fig. 4. Vertical distribution of *in vivo* fluorescence, soluble fluorescence, and percentage soluble fluorescence in two hydrological situations: upper figure, upwelling situation; lower figure, tropical situation: units of fluorescence are the arbitrary units of fluorometer on 30× scale, with the most sensitive door.

Such, including phaeophorbides as well as a variety of unknown decomposition products, are commonly called phaeophytin (Holm-Hansen *et al.*, 1965). Since the SF indicates the presence of phaeopigments released by cells and since the phaeophytin indicates the presence of the same products in the cell, there should be a correlation between the two. Such correlation is shown in Fig. 5 ($r = 0.81$).

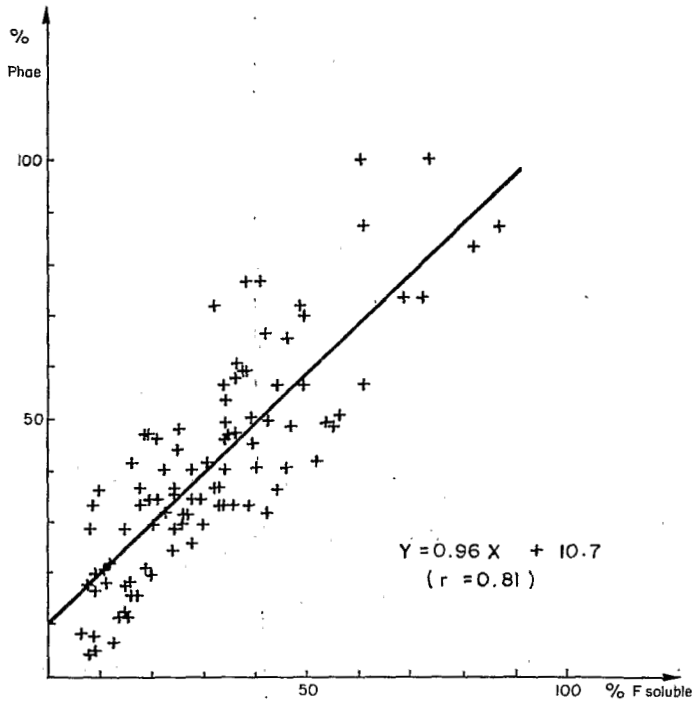

Fig. 5. Linear regression between the percentage of soluble fluorescence, and the percentage of phaeophytin: $\hat{y} = 0.96\,x + 10.7$ ($n = 104$ pairs).

The regression equation is,

$$\hat{y} = 0.96\,x + 10.7,$$

where x is the percentage of SF and y the percentage of phaeophytin.

Lorenzen (1967) considered that the phaeopigments in the water column are the residue of zooplankton grazing and the amount of phaeophytin is a measure of grazing pressure on existing phytoplankton populations. From our results, it appears that the grazing pressure on existing phytoplankton populations would be higher in the mixed layer of the tropical ocean than in the thermocline. It is, however, difficult to distinguish between the effect of grazing and degeneration due to the limited nutrients.

This new technique, examined in the oligotrophic open sea of the Atlantic Ocean

is simple and very sensitive. It could be used for continuous recording, with an on-line filtration system, simultaneously with *in vivo* F during a surface survey or a vertical exploration. In the oceanic ecosystems it gives valuable and rapid information on the degree of maturation of the phytoplankton populations including the physiological state of the cells and the grazing pressure. It could be applied to different waters, but it must be remembered that in coastal or inland waters, interference due to terrestrial or benthic vegetal detritus will increase. In these waters SF is an indicator of exogenous organic matter and the *in vivo* F will have little value for determining the amount of phytoplankton pigments. By subtracting the SF from the *in vivo* F we obtain 'extracted fluorescence values' which are well correlated with the chlorophyll *a* values (Dufour, pers. comm.).

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