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THE SIZE STRUCTURE AND METABOLISM OF THE PELAGIC ECOSYSTEM. *J. RODRÍGUEZ* and *W. K. W. LI (eds.)*.

Phytoplankton biomass and chlorophyll concentration across the North Atlantic*

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SUMMARY: The bulk concentration of phytoplankton chlorophyll and carbon may be represented in proxy by summations derived from flow cytometric spectra of cellular fluorescence and cellular forward light scatter.Changes in the bulk properties reflect changes in the adaptive properties of cells (cellular content of chlorophyll and carbon) as well as the weighting factor which is the cell abundance. In coastal waters off Morocco, depth profiles of total fluorescence and total forward light scatter matched those of bulk chlorophyll, suggesting a relative invariance of the carbon : chlorophyll ratio with depth.In the oceanic central North Atlantic, depth profiles of bulk chlorophyll were matched by those of total fluorescence but not by those of total forward light scatter.Phytoplankton in the subsurface chlorophyll maximum of oceanic waters are thus inferred to have a substantially lower carbon : chlorophyll ratio than phytoplankton near the surface.In the oceanic North Atlantic, maxima in ultraphytoplankton abundance and light scatter are located at depths shallower than the maximum in chlorophyll.

Key words: Chlorophyll, fluorescence, flow cytometry, light scatter, phytoplankton.

RESUMEN: BIOMASA PITOPLANCTÓNICA Y CONCENTRACIÓN DE CLOROFILA EN EL ATLÁNTICO NORTE. — La concentración de clorofila y carbono fitoplanctónico puede ser representada por sumatorios derivados de espectros de fluorescencia celular y dispersión frontal de luz obtenidos mediante citometría de flujo. Cambios en las propiedades globales reflejan cambios en las propiedades adaptativas de las células (contenido celular de clorofila y carbono) así como en el factor de ponderación que constituye la abundancia celular. En las aguas costeras de Marruecos, los perfiles verticales de fluorescencia total y dispersión frontal total de la luz se ajustan a los de clorofila, lo que sugiere una relativa constancia de la relación carbono: clorofila con la profundidad. En las aguas centrales del Atlántico Norte, los perfiles verticales de clorofila coinciden con los de fluorescencia total pero no con los de dispersión frontal total de luz. Se deduce por tanto que el fitoplancton a nivel del máximo subsuperficial de clorofila en aguas oceánicas tiene una relación carbono: clorofila sustancialmente inferior que el fitoplancton cerca de la superficie. En el Atlántico Norte, los valores máximos de abundancia de ultrafitoplancton y de dispersión de la luz se sitúan a profundidades menores que el máximo de clorofila.

Palabras clave: Clorofila, fluorescencia, citometría de flujo, dispersión de luz, fitoplancton.

INTRODUCTION

The standing stock of chlorophyll *a*, CHL (μg Chl l^{-1}) and of phytoplankton carbon, *C* (μg C l^{-1}) are two bulk properties of fundamental importance in biological oceanography. Both quantities depend on the amount of living algal matter, but they do not necessarily convey the same information because the *C*:CHL ratio is variable (CULLEN, 1982; GEIDER, 1987). Estimates of *C* are difficult to obtain because unlike CHL, there is no direct method for a bulk chemical assay of *C* (EPPLEY *et al.*, 1977).Studies such as that of FURUYA (1990) in which vertical profiles of CHL are compared with those of microscope-

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derived values of C are few, yet of crucial importance (LONGHURST and HARRISON, 1989).

In this paper, I use an approach whereby C and CHL are represented by proxy variables derived from the frequency distributions of phytoplankton cell size and pigment content. This approach, recently applied in a slightly different form to study ultraphytoplankton in the eastern Mediterranean Sea (LI *et al.*, 1993), is here used for flow cytometric data collected across the North Atlantic Ocean during late summer-early fall. The purpose is to infer, for different regions, whether the depth variation of CHL reflects that of C. There is much recent evidence that the vertical structure of total particulate concentration (as indicated by beam attenuation) does not match that of CHL in open ocean waters (PAK *et al.*, 1988; KITCHEN and ZANEVELD, 1990). However there is inadequate information on the elements underlying these bulk properties: which are, on the one hand, the optical and physiological properties of each cell, and on the other hand, the abundance of these cells (but see MOREL *et al.*, 1993). In this paper, the bulk properties C and CHL are examined in terms of the light scattering and fluorescence from individual phytoplankters, together with the abundance of these cells in seawater.

CONCEPTUAL OUTLINE

A compelling goal in biological oceanography is to demonstrate that bulk properties of the phytoplankton (such as CHL and C) can be derived from the combined contribution of constituent cells (PLATT, 1989; YENTSCH and CAMPBELL, 1991). On this basis, we recognise that the standing stocks of CHL (μg Chl l^{-1}) and C (μg C l^{-1}) at depth z (m) can be expressed as:

$$\mathrm{CHL}(z) = \sum_i n_i(z)\cdot chl_i(z) \qquad (1)$$

$$C(z) = \sum_i n_i(z)\cdot c_i(z) \qquad (2)$$

where subscript i represents a class interval for pigment content or size, n is the phytoplankton abundance (cells l^{-1}), *chl* is the cellular content of chlorophyll a (μg Chl $cell^{-1}$), and c is the cellular content of carbon in phytoplankton (μg C $cell^{-1}$). This approach, in particular Eq. 2, is simply a formal statement of the principle upon which conventional microscopic methods are based (e.g. BOOTH, 1988; FURUYA, 1990).

Direct implementation of Eqs. 1 and 2 is difficult because *chl* and c cannot be easily or precisely quantified in natural samples. Instead, we substitute two related variables that can be measured with ease and precision: f = chlorophyll fluorescence per cell, and s = light scatter per phytoplankton cell. The rationale for these substitutions is that fluorescence depends on chlorophyll content (SOSIK *et al.*, 1989), and light scatter depends on cell volume which is related to carbon content (CHISHOLM, 1992).

$$\mathrm{CHL}(z) = \sum_i n_i(z)\cdot f_i(z)\cdot \psi_i(z) \qquad (3)$$

$$C(z) = \sum_i n_i(z)\cdot s_i(z)\cdot \chi_i(z) \qquad (4)$$

where ψ is the chlorophyll content per unit fluorescence, and χ is the carbon content per unit light scatter for phytoplankton. Admittedly, light scatter depends also on the shape and refractive index of the cell so that an interpretation of scatter intensity in terms of relative carbon content cannot, in principle, be robust.Nevertheless, OLSON *et al.* (1989) have demonstrated a strong empirical correlation between forward light scatter and Coulter volume using a wide variety of algae including calcifying strains of coccolithophores. These authors suggested that at the low angles used by the flow cytometer to measure forward scatter, the influences of cell shape and refractive index are minor.

Proceeding from Eqs. 3 and 4, it is necessary to introduce a further simplification because $\psi_i(z)$ and $\chi_i(z)$ are not known.We make the assumptions (to be discussed later) that $\psi_i(z)$ and $\chi_i(z)$ are the same for all i; they are therefore replaced by the quantities $\Psi(z)$ and $\mathrm{X}(z)$.

$$\mathrm{CHL}(z) = \Psi(z).\left[\sum_i n_i(z)\cdot f_i(z)\right] \qquad (3)$$

$$C(z) = \mathrm{X}(z)\cdot\left[\sum_i n_i(z)\cdot s_i(z)\right] \qquad (4)$$

Given the assumption that $\Psi(z)$ is constant, Eq. 5 shows that $\Sigma n_i(z)\cdot f_i(z)$ is a relative measure of CHL (z). Likewise, with $\mathrm{X}(z)$ constant, Eq. 6 shows that $\Sigma n_i(z)\cdot s_i(z)$ is a relative measure of $C(z)$.

For notational simplicity, we establish the following assignments:

$$F(z) = \sum_i n_i(z)\cdot f_i(z) \qquad (7)$$

$$S(z) = \sum_i n_i(z)\cdot s_i(z) \qquad (8)$$

$$N(z) = \sum_i n_i(z) \qquad (9)$$

where $F(z)$ is the total fluorescence, $S(z)$ is the total scatter, and $N(z)$ is the total cell abundance.

The mean fluorescence per cell, $\bar{f}(z)$ and the mean scatter per cell, $\bar{s}(z)$ are given simply by:

$$\bar{f}(z) = \frac{F(z)}{N(z)} \tag{10}$$

$$\bar{s}(z) = \frac{S(z)}{N(z)} \tag{11}$$

Our goal is to relate the depth distribution of CHL (z) with that of $C(z)$. We do this by considering the proxy variables $F(z)$ and $S(z)$ according to Eqs. 7 and 8. The required frequency distributions of $f_i(z)$ and $s_i(z)$ are easily constructed from flow cytometric measurements of phytoplankton abundance, $n_i(z)$. The approach taken here is an ataxonomic one. In other words, the categories represented by i classes do not have names; instead, they are the relative intensities of cellular fluorescence and cellular light scatter.

MATERIAL AND METHODS

Seawater samples were collected using Niskin bottles during cruise 92-037 of CSS *Hudson*. The cruise consisted of three portions (Fig. 1): stations 1-20 covered the eastward transect from Halifax (Canada) to Las Palmas (Canary Islands) from September 18 to 26, 1992; stations 22-54 were located near the coast of Morocco from September 28 to October 7; and stations 57-85 covered the westward transect from Las Palmas to Halifax from October 9-19.

Measurements of cell abundance, fluorescence per cell, and forward (narrow angle) light scatter per cell were obtained by flow cytometry (FACSort™, Becton Dickinson). Samples of 0.25-0.50 ml were freshly analysed after removal from the Niskin bottles. Fluorescence at wavelengths longer than 650 nm (488 nm excitation) was ascribed to chlorophyll *a*. Light scattered over a range of half-cone angles from <1 to 10 degrees was collected by a solid state silicon detector with spectral response from 300 to 1100 nm (specifications from manufacturer). To ensure that only phytoplankton were enumerated, particles with red fluorescence weaker than that emitted by *Prochlorococcus* were rejected. The threshold for rejection was established after *Prochlorococcus* had been identified from cytometric signatures (LI, 1994). Measurements of fluorescence and light scatter were collected using logarithmic amplification and recorded in relative units in a 4-decade range spanned by $i = 256$ channels. Data were extracted from listmode format using LYSYS™ II software (Becton Dickinson). Relative intensities of fluorescence and scatter were converted to respective linear scales and ex-

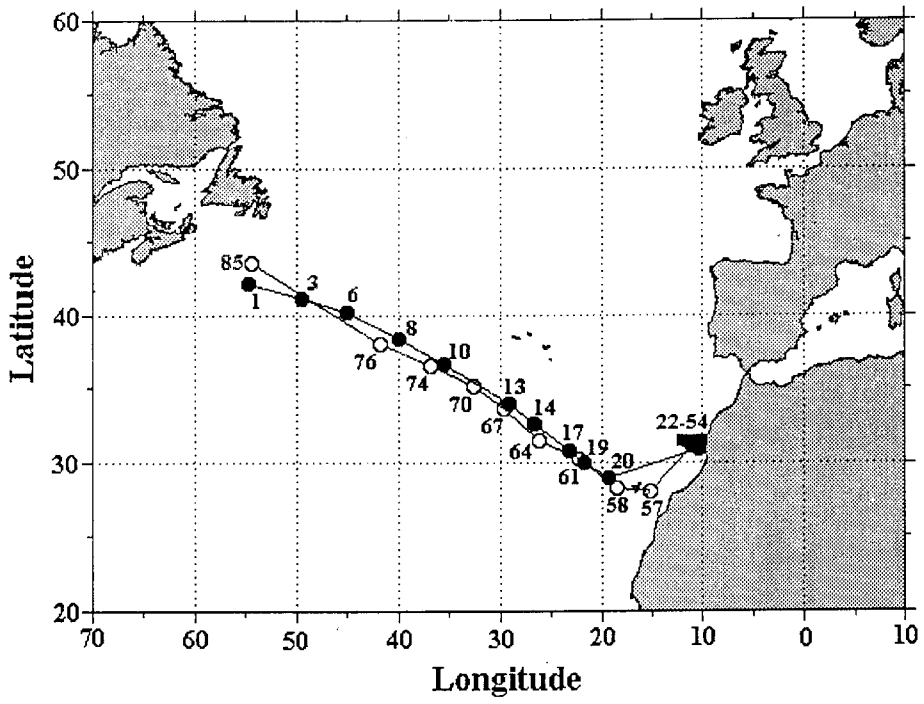

FIG. 1. — Locations where samples were collected for shipboard flow cytometric analysis: eastward transect (○); westward transect (●); near Morocco (■).

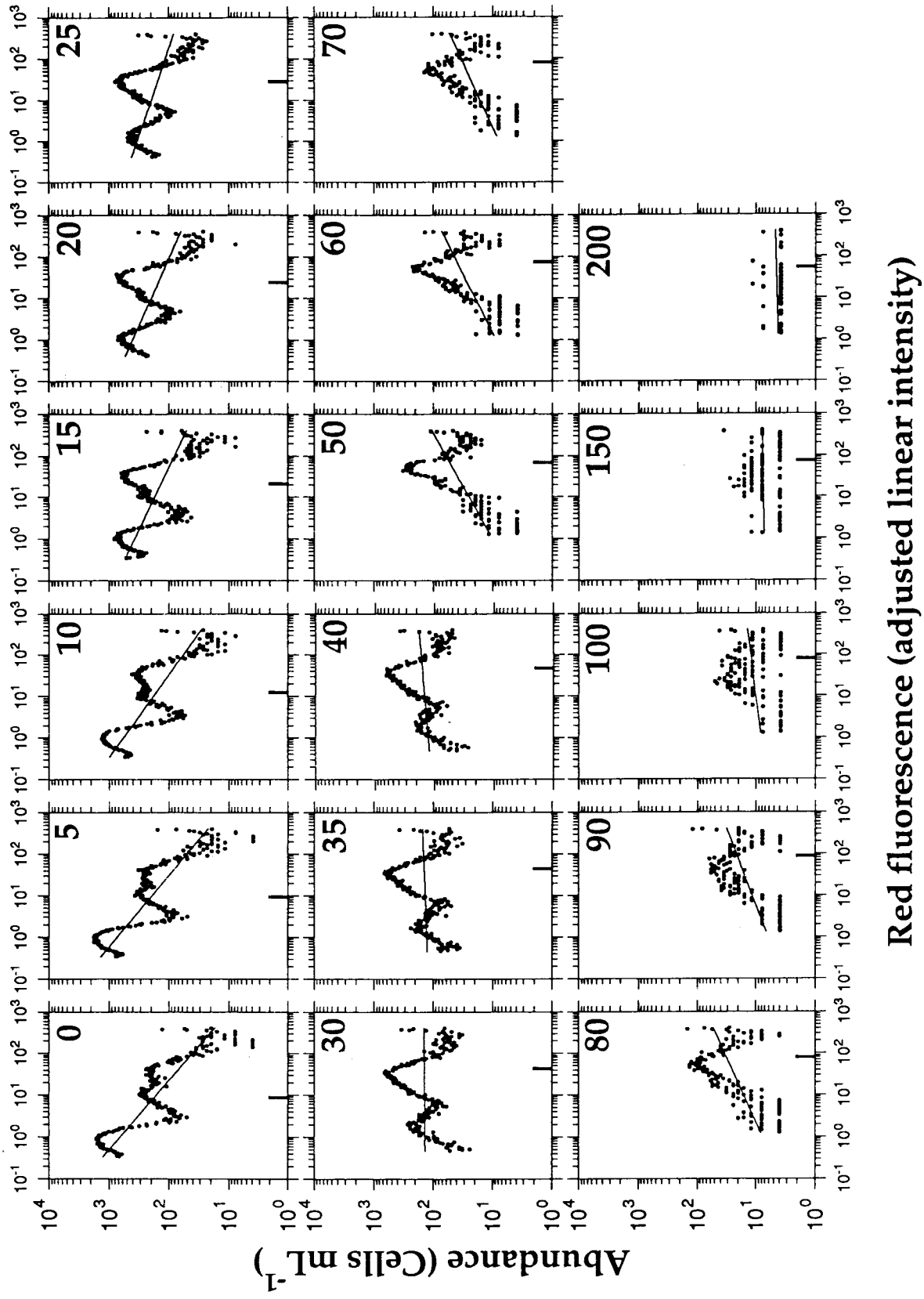

FIG. 2. — Numerical abundance (n_i, cells ml^{-1}) of ultraphytoplankton at station 30 distributed across a range of chlorophyll fluorescence intensity (f_i, ALI). Depth (m) of the sample is indicated in the upper right of each panel; mean ALI (Eq. 10) is indicated by the short vertical bar at the bottom of each panel; sloping straight line through the data indicates linear regression.

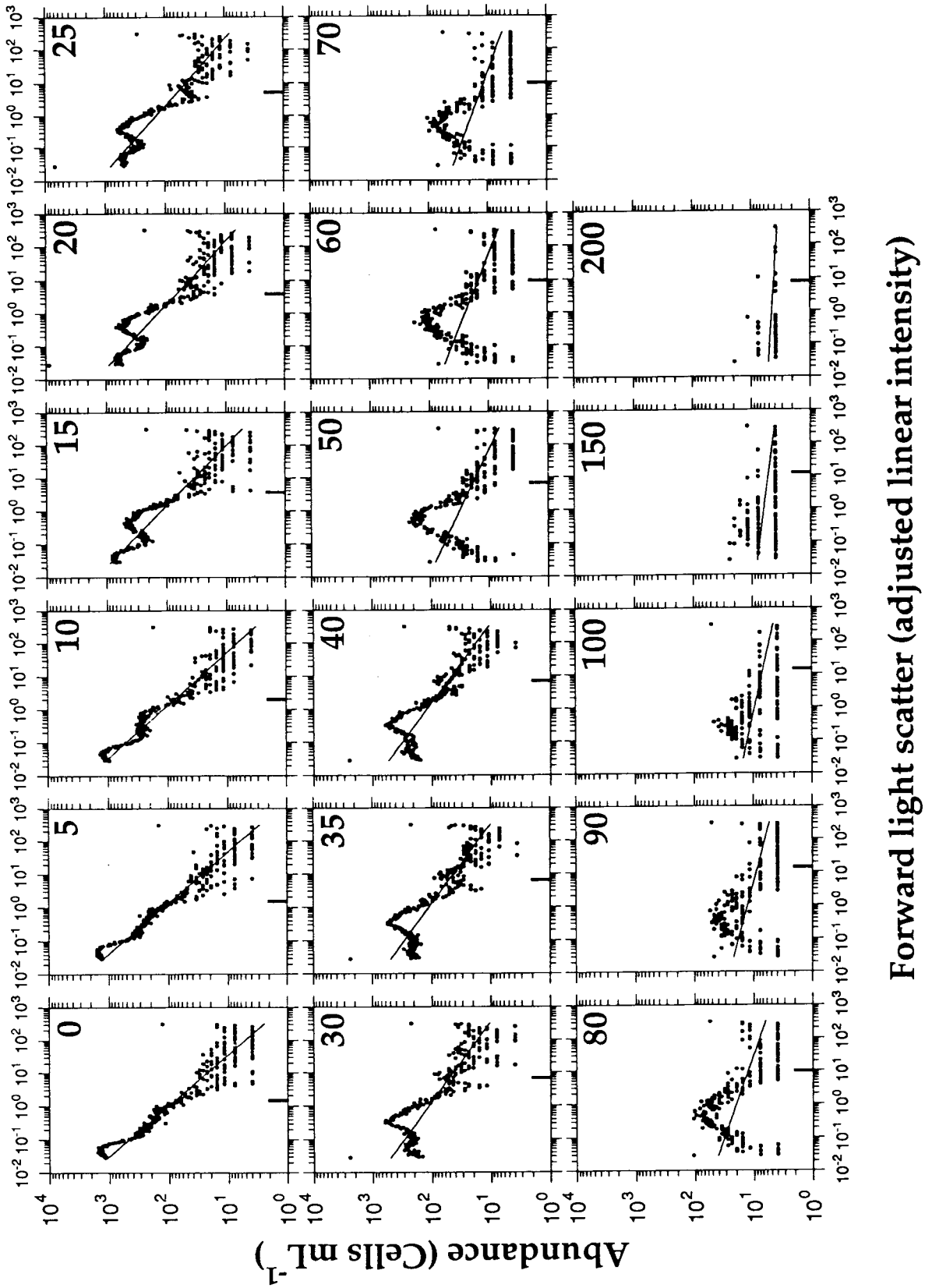

FIG. 3. — As for Fig. 2 except that abundance is distributed across a range of forward light scatter intensity (s_i, ALI). Mean ALI (Eq. 11) is indicated by the short vertical bar at the bottom of each panel.

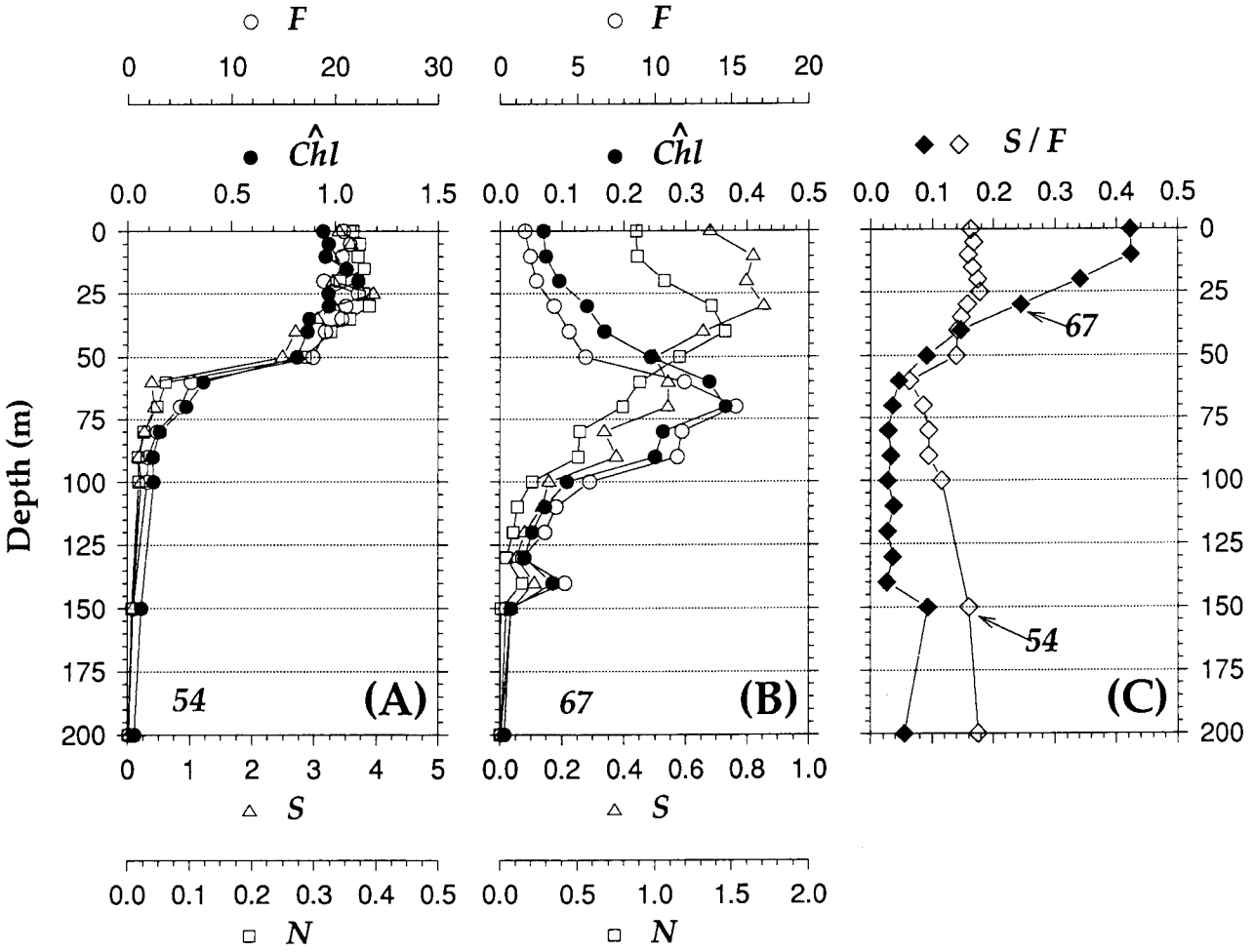

FIG. 4. — (A,B) Contrast between coastal station 54 and open ocean station 67. $\hat{Chl}$ (●, μg Chl l^{-1}); N (□, 10^8 *cells* l^{-1}); F (○, 10^8 cells l^{-1} · ALI); S (△, 10^8 cells l^{-1} · ALI). (C) The ratio of S : F for the coastal station 54 (◇) and the open ocean station 67 (◆).

pressed as "Adjusted Linear Intensities" (ALI) following the procedure of HORAN *et al.* (1990).

Bulk chlorophyll a was measured (Turner 10-AU fluorometer) on acetone extracts of particulate matter collected on glass fibre filters (Whatman GFF). This measured quantity is denoted as $\hat{Chl}$.

RESULTS

Frequency Distributions

The distributions of n_i over f_i (Fig. 2) and n_i over s_i (Fig. 3) were polymodal, indicating differential contribution from various kinds of cells (*Prochlorococcus, Synechococcus*, and diverse eukaryotic ultraphytoplankters). With increasing depth, cells that were more fluorescent and more highly-scattering became relatively more important. This can be seen as an increase in the mean of the distributions $(\bar{f}(z), \bar{s}(z))$ and also as an increase in the slope (i.e. more positive) of the spectra (Figs. 2,3).

Depth Profiles

In nutrient-rich coastal waters (e.g. sta 54, Fig. 4A), $F(z)$, $S(z)$, $N(z)$and $\hat{Chl}(z)$ all varied with depth in the same manner: values were high in the upper layer, decreasing sharply at the pycnocline. On the other hand, in oceanic waters (e.g. sta 67, Fig. 4B), a different situation prevailed. Whereas $F(z)$ and $\hat{Chl}(z)$ displayed pronounced deep subsurface maxima (*ca* 70 m), $S(z)$ and $N(z)$ manifested less pronounced maxima at significantly shallower depths (*ca* 30-40 m).

The ratio of $S(z)$: $F(z)$, which might be considered a proxy of C : CHL, differed only slightly through the euphotic zone in coastal waters, but decreased sharply with depth in oceanic waters (Fig. 4C). Recognising that $S(z)$: $F(z)$ is equivalent to

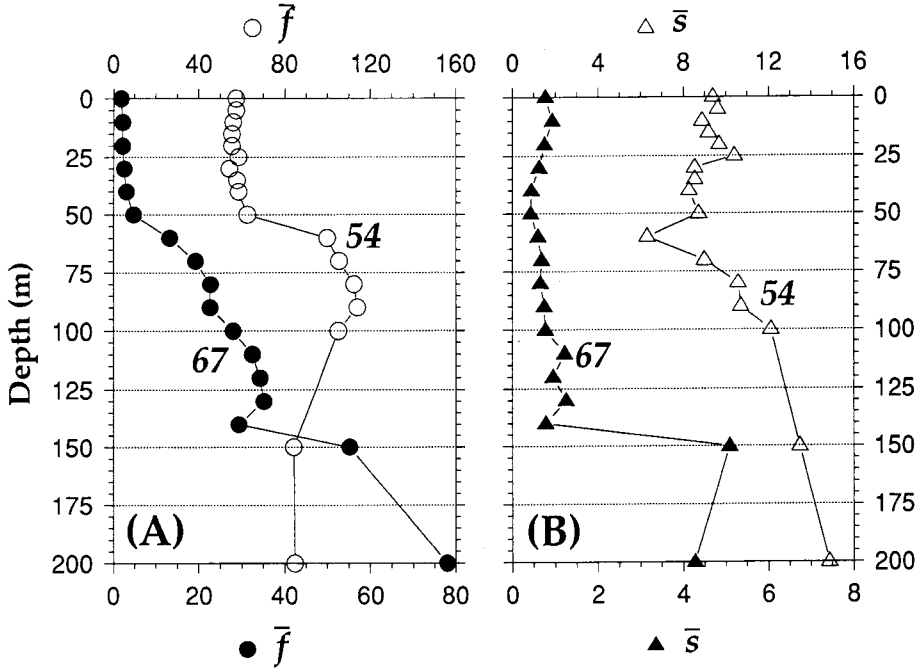

FIG. 5. — (A) Mean fluorescence per cell ($\bar{f}$) and (B) mean light scatter per cell ($\bar{s}$) at coastal station 54 (○,Δ) and at open ocean station 67 (●, ▲). Measurement unit for all quantities is ALI. Note that there is a two-fold difference in the abscissa scales for station 54 (upper axis) and for station 67 (lower axis).

$\bar{s}(z) : \bar{f}(z)$, we show that most of the vertical variation in $S(z) : F(z)$ was associated with larger relative change in $\bar{f}$(z) at depth (Fig. 5). Not surprisingly, $\bar{f}$(z) and $\bar{s}$(z) were much higher for coastal than oceanic phytoplankton (Fig. 5).

North Atlantic Transect

The transect covered three major regions: the Gulf Stream at the western end, the upwelling off Morocco at the eastern end, and the central North Atlantic in the middle (Fig. 6, upper panels). Across the entire transect, $F(z)$ was a good descriptor of $\hat{Chl}(z)$ (Fig. 7).

For $S(z)$, the depth distribution was similar to that of $\hat{Chl}(z)$ only at the Moroccan upwelling and at the Gulf Stream. Across the central North Atlantic, the weak subsurface maximum of $S(z)$, where present, was significantly shallower than the pronounced deep subsurface maximum of $\hat{Chl}(z)$ (Fig. 7).

The trans-Atlantic section of $N(z)$ resembled that of $S(z)$ except at the Moroccan upwelling where total cell abundance was low (Fig. 7).

More detailed information was obtained from an inshore-to-offshore transect near Morocco consisting of 13 profiles (stations 32-44) located approximately 10 min longitude apart (Fig. 6, lower panels). This section documented the transition from coastal to oceanic conditions. At stations east of ca 10.5° W near Morocco, highest values of $F(z)$, $S(z)$, and $\hat{Chl}(z)$ were attained in the upper layer (Fig. 8). Station 54 (Fig. 4A) was representative of this situation. In contrast, stations west of 10.5° W showed emerging characteristics of the oceanic situation (Fig. 8) represented by station 67 (Fig. 4B): that is, subsurface maxima of $F(z)$ and $\hat{Chl}(z)$.

F, S, $\bar{f}$ and $\bar{s}$ versus $\hat{Chl}$

The proxy variable F was clearly a good indicator of $\hat{Chl}$. The relationships between F and $\hat{Chl}$ established for oceanic data (Fig. 9A) and for coastal data (Fig. 10A) were statistically indistinguishable ($P > 0.25$).

The proxy variable S, which is used to indicate C, was well correlated with $\hat{Chl}$ in coastal waters (Fig. 10B) but not in oceanic waters (Fig. 9B). In the latter cases, near-surface phytoplankton (plotted as filled triangles) scattered more light per unit $\hat{Chl}$ than deeper phytoplankton.

The mean values, $\bar{f}$ and $\bar{s}$, were not correlated with $\hat{Chl}$ (Figs. 9C,D; 10C,D) except in the case of near-surface coastal phytoplankton ($P < 0.05$, Fig. 10C,D).

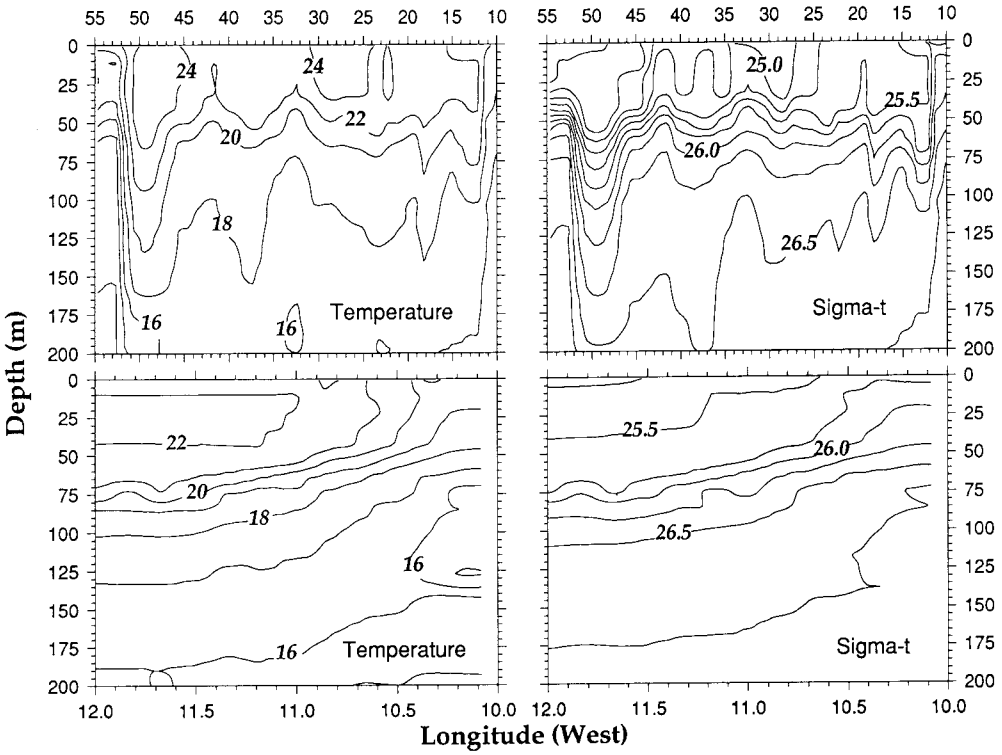

FIG. 6. — Sections of temperature and sigma-t across the North Atlantic (upper panels).Detailed sections constructed from an inshore-to-offshore transect near Morocco consisting of 13 profiles (stations 32-44) located approximately 10 min longitude apart (lower panels).

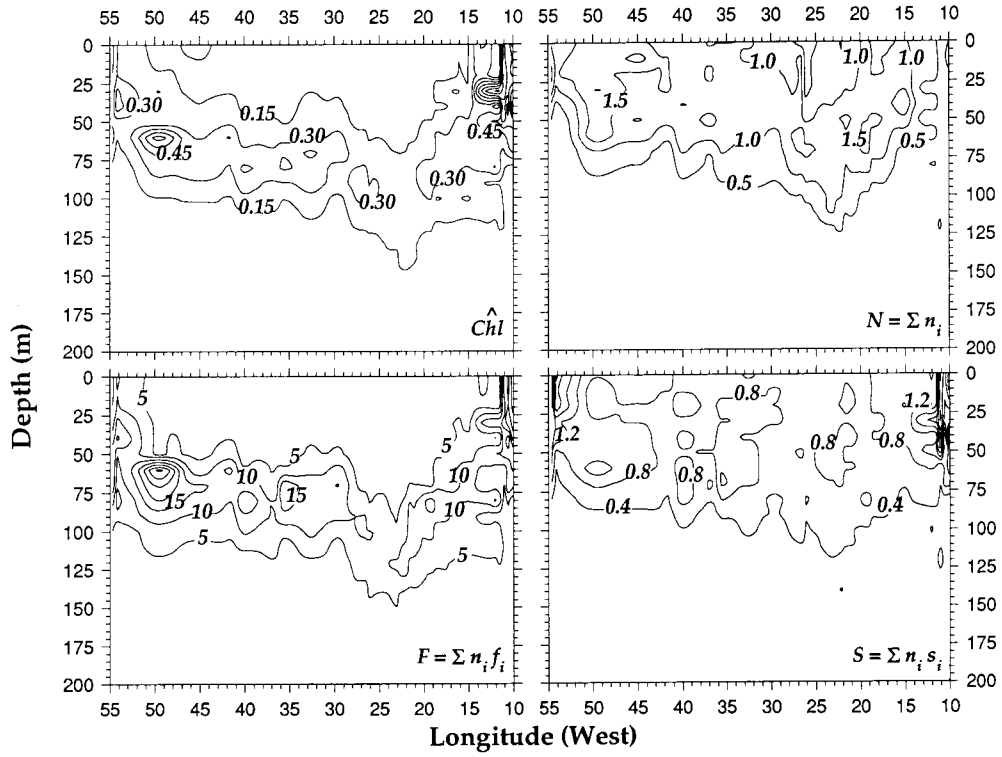

FIG. 7. — The distribution of variables across the North Atlantic. $\hat{Chl}$(μg Chl l^{-1}); N (10^8 cells l^{-1}); F (10^8 cells l^{-1} · ALI); S (10^8 cells l^{-1} · ALI).

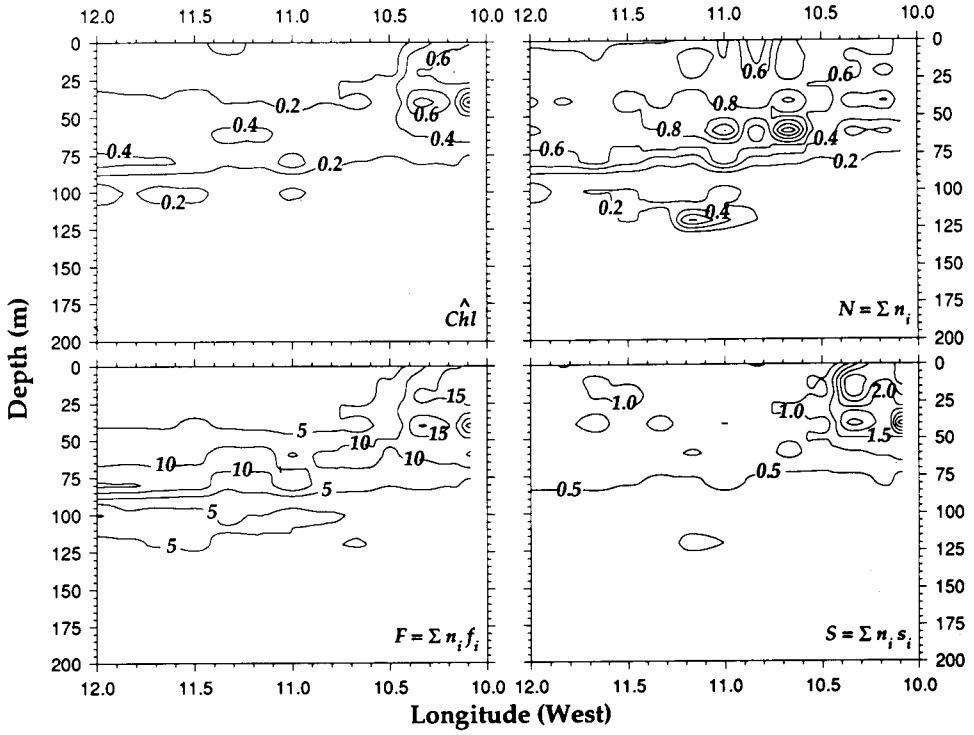

FIG. 8. — The distribution of variables along an inshore-to-offshore transect near Morocco. $\hat{Chl}$(μg Chl l^{-1}); N (10^8 cells l^{-1}); F (10^8 cells l^{-1} · ALI); S (10^8 cells l^{-1} · ALI).

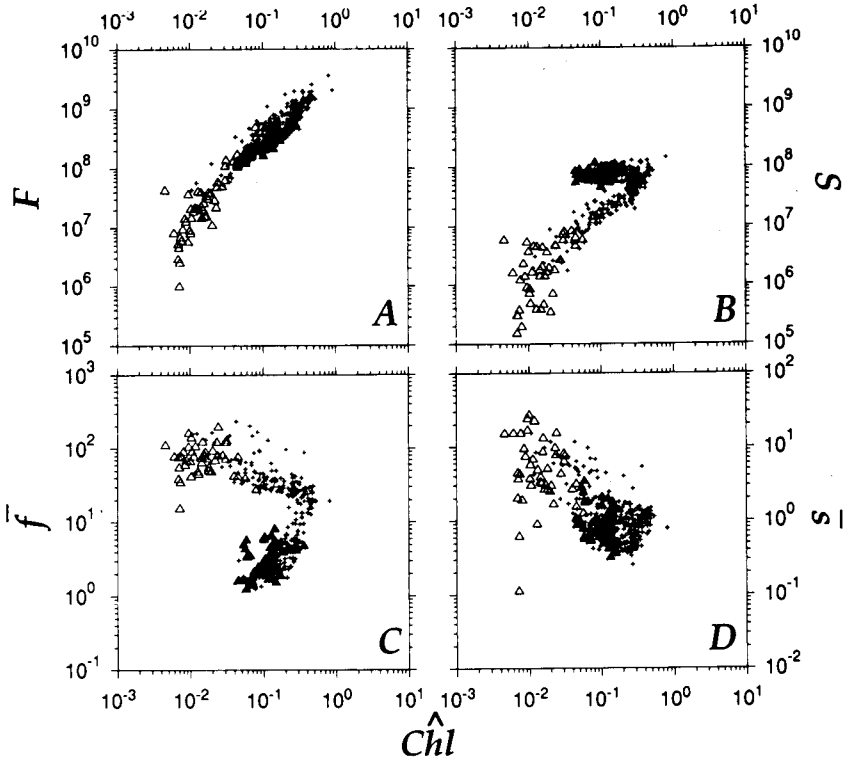

FIG. 9. — Central North Atlantic: the relationship between measured chlorophyll $\hat{Chl}$, μg l^{-1} and (A) total fluorescence F, [cells l^{-1}] · ALI, (B) total scatter S, [cells l^{-1}] · ALI, (C) mean fluorescence per cell (f, ALI, (D) mean scatter per cell $\bar{s}$, ALI. Samples at $z \leq 20$ m (▲); at $z \geq 150$ m (Δ); at 20 m < z < 150 m (+).

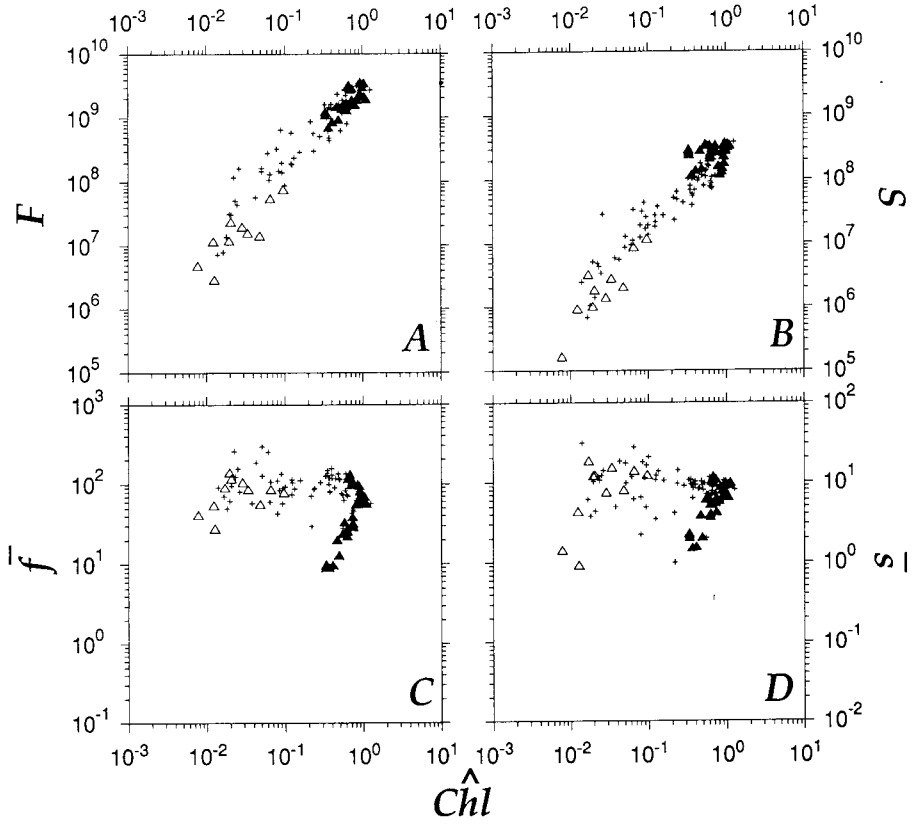

FIG. 10. — As for Fig. 8 except that the data are from off the Moroccan coast and at the Gulf Stream.

DISCUSSION

Throughout the oceanic central North Atlantic in late summer-early fall, the vertical distributions of F and S are here shown to be different — the maximum of F being deeper than the maximum of S. By implication, the vertical distributions of CHL and C were also different. In contrast, the vertical distibutions of F and S (thus also CHL and C) were the same in nutrient-rich coastal waters. These results confirm a generalisation recently expressed by LONGHURST and HARRISON (1989): namely that "the SCM (depth of subsurface chlorophyll maximum layer) is coincident with algal biomass when it is at a depth where neither light nor nutrients are limiting. But, where it is sufficiently deep that light becomes limiting, the biomass maximum will be shoaler than the SCM".

To put it another way, it can be said that the C: CHL ratio decreases with depth in the oceanic central North Atlantic. Hitherto, the SCM has been represented as a layer where CHL is high because of physiological adaptation to low irradiance and greater availability of nutrients (STEELE, 1964; CULLEN, 1982). With flow cytometric measurements made on single cells, it is now possible to be more specific about the changes in the bulk ratio C: CHL. In particular, it is evident that changes in the bulk properties reflect changes in the adaptive cellular properties *(chl, c)* as well as the weighting factor which is the cell abundance *(n)*.

Measurements of the variables F and S are easily made and afford us an extensive view of the ocean (Figs. 7,8). Moreover, spectra related to pigment (Fig. 2) and size (Fig. 3) contain important information (CHISHOLM, 1992) concerning the flow of biomass through pelagic ecosystems (PLATT and DENMAN, 1977; RODRIGUEZ and MULLIN, 1986). Integration of the spectra yields quantities related to bulk properties (e.g. DEMERS *et al.*, 1989), leading to a characterisation of the assemblage as a whole from the details of the components. However, it is explicitly clear that the usefulness of F and S as proxies for CHL and C depends on the extent to which ψ_i and χ_i are the same for all i (Eqs. 5,6). The values of ψ_i and χ_i for natural phytoplankton are not known, but there is evidence from laboratory cultures that both param-

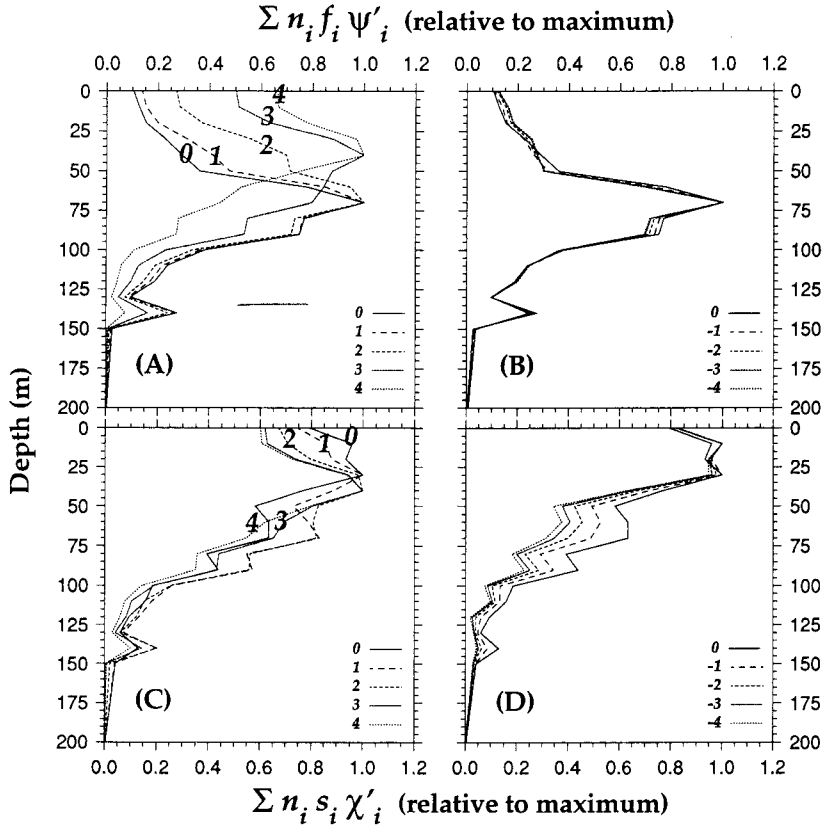

FIG. 11. — Effects of varying ψ'_i and χ'_i at station 67. The number labelling each profile is x, where $\psi'_0/\psi'_{255} = 10^x$ or $\chi'_0/\chi'_{255} = 10^x$. (A) $\Sigma\ n_i \cdot f_i \cdot \psi'_i$ for $x \geq 0$, (B) $\Sigma\ n_i \cdot f_i \cdot \psi'_i$ for $x \leq 0$, (C) $\Sigma\ n_i \cdot s_i \cdot \chi'_i$ for $x \geq 0$, (D) $\Sigma\ n_i \cdot s_i \cdot \psi'_i$ for $x \leq 0$. Note that when $x = 0$, ψ'_i and χ'_i are the same for all i, giving the results indicated by F and S respectively as shown in Fig. 4B.

eters vary with species and physiological conditions (SOSIK *et al.*, 1989; CHISHOLM, 1992).

The question we face is the following. Since ψ_i and χ_i are likely not the same for all i, how much difference in these parameters can be tolerated without invalidating our major conclusion: namely that the maximum of CHL is deeper than the maximum of C in the oceanic central North Atlantic? To examine this question, it is necessary only to know the values of CHL(z) and $C(z)$ in a relative sense, that is normalised to their respective maxima. For this purpose, relative values of $\psi'_i(z)$ and $\chi'_i(z)$ are defined to vary in logarithmic fashion over i:

$$\psi'_i(z) = \chi'_i(z) = 10^{\left(\frac{255-i}{255}\right)\cdot x}$$

The desired normalised values, CHL$'(z)$ and $C'(z)$ can be obtained using $\psi'_i(z)$ and $\chi'_i(z)$ in place of their respective absolute equivalents in Eqs. 3 and 4. The dynamic range spanning classes $i = 0$ to 255 is given by $10^{|x|}$. The parameters ψ'_i and χ'_i decrease with i for $x > 0$, increase with i for $x < 0$, and remain constant for $x = 0$. The third case is equivalent to the representation of Eqs. 5 and 6.Using the data from oceanic station 67 (*cf* Fig. 4B), we calculated profiles of CHL$'(z)$ and $C'(z)$ for x from –4 to 4 (Fig. 11). The analysis indicated firstly that $C'(z)$ was largely insensitive to changes in χ'_i (Fig. 11C,D). Secondly, CHL$'(z)$ was sensitive to changes in ψ'_i only when $x > 0$ (Fig. 11A,B). In considering the depth of the SCM, we found no shallowing when $x < ca$ 2.7. In other words, a difference of less than 500-fold (i.e. $10^{2.7}$) between ψ'_0 and ψ'_{255} did not alter the depth of the SCM. For the SCM to have occurred at 30 m where $C(z)$ apparently attained its maximum value, a difference of about 25,000-fold (i.e. $10^{4.4}$) between ψ'_0 and ψ'_{255} would be required. We are not aware of evidence confirming or refuting a variation of this magnitude in the fluorescence yield of chlorophyll from ultraphytoplankton. For now, we suggest that the maximum of CHL is indeed deeper than the maximum of C in the oceanic central North Atlantic.

The relationship between F and $\hat{Chl}$ (Figs. 9A, 10A) appears to be robust. Supporting results are available from both LI *et al.* (1993) and SHIMADA *et*

al. (1993) who partitioned chlorophyll fluorescence amongst *Prochlorococcus, Synechococcus,* and small eukaryotic algae. In these studies, profiles of F and $\hat{Chl}$ also matched very well. For some oceanographic purposes (e.g. locating the SCM), it seems justified to use the information-rich quantity F as a proxy for $\hat{Chl}$.

The major goal of our work was to examine the relationship between C and CHL. To this end, it is the scatterplots of $S(z)$ vs $\hat{Chl}(z)$ (Figs. 9B, 10B) which are most relevant. With the one important exception of near-surface phytoplankton in oceanic waters (Fig. 9B), there was an impressive correlation between $S(z)$ and $\hat{Chl}(z)$. The remarkable nature of this relationship is underscored by an apparent lack of association between $\bar{s}(z)$ and $\hat{Chl}(z)$ (Figs. 9D, 10D). If however, consideration is given only to near-surface phytoplankton, it is clear that coastal cells have higher $\bar{s}$ than oceanic cells — concurring with a widely-held notion that larger cells are more prevalent in coastal waters because of greater nutrient availability (YENTSCH and PHINNEY, 1989). Across the central oceanic North Atlantic, however, the relationship between $S(z)$ and $\hat{Chl}(z)$ is complicated by the effects of environmental adaptation which uncouple the cellular syntheses of carbon and chlorophyll. As a consequence, depth profiles of C do not match those of CHL in these regions.

Not surprisingly, this conclusion can also be drawn from similar measurements made at three depths (2, 40 and 80 m) at the OFP station (31°50′N, 64°10′W) off Bermuda (CHISHOLM, 1992) where the CHL maximum was located at 80 m. From these data, we were able to compute $F(z)$ (Eq. 7) and also $C(z)$ (Eq. 2). The results are $F(2) = 1.8 \times 10^5$, $F(40) = 2.8 \times 10^5$, $F(80) = 11.1 \times 10^5$ relative units; and $C(2) = 15$, $C(40) = 21$, $C(80) = 17$ μg C l^{-1}. Thus, off Bermuda, the CHL maximum was also the maximum for total fluorescence F, but not for phytoplankton carbon biomass C. The data of CHISHOLM (1992) are more refined than ours in two ways. First, the flow cytometric light scatter measurements (s_i) were converted to values of cellular carbon (c_i) by appropriate calibrations, thus enabling the calculation of C (Eq. 2) instead of S (Eq. 8). Second, the range of measurements was extended to include phytoplankton cells larger than the ultraplankton. This was achieved by hardware modification designed for more rapid sample throughput, allowing the less abundant nanoplankton to be counted in sufficient numbers. In view of the similar conclusion about the mismatch of $C(z)$ and $\hat{Chl}(z)$, it seems that our less refined analysis possesses a satisfactory degree of robustness.

The difference between $S(z)$ (or $C(z)$) and $F(z)$ (or $\hat{Chl}(z)$) described above is similar to recent results concerning the relationship between the concentration of total microparticles and bulk chlorophyll fluorescence. These latter results, obtained by measurements of the beam attenuation coefficient at 665 nm and measurements of in situ fluorescence, indicate that the maximum particle concentration generally occurs at a shallower depth than the maximum chlorophyll concentration in oceanic waters (PAK *et al.*, 1988; KITCHEN and ZANEVELD, 1990). Our flow cytometric results, which refer exclusively to particles exhibiting red fluorescence (i.e. phytoplankton), are presented in the familiar Λ (inverted V) plot (DENMAN and GARGETT, 1988; KITCHEN and ZANEVELD, 1990; HOEPFFNER and SATHYENDRANATH, 1992; WEEKS *et al.*, 1993) to indicate the non-correlation between attenuance and fluorescence signals (Fig. 12). In common with Λ plots based on total microparticles, Fig. 12 shows an inverted V whose left leg represents samples below the chlorophyll maximum and whose right leg those above it. This summary of data from 27 profiles shows a linear relationship between F and S below the chlorophyll maximum but a lack of correlation between the variables above the chlorophyll maximum. Since our data refer only to the phytoplankton and not to total microparticles, it is evident that there should be no significant positive

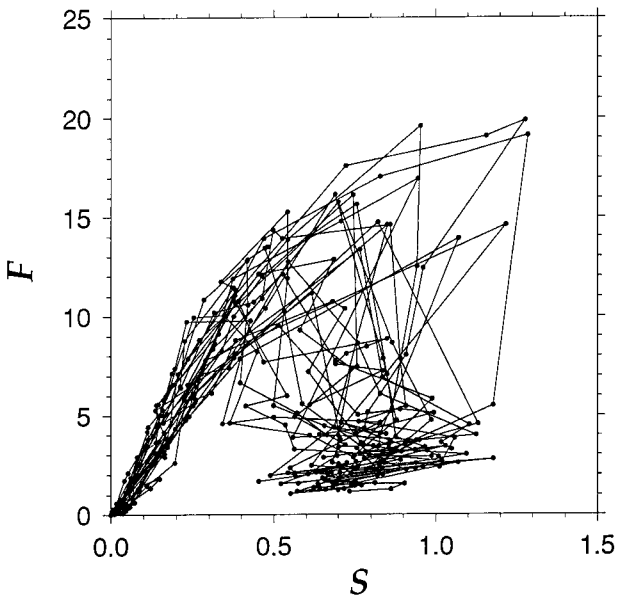

FIG. 12. — The variation of $F(z)$ with $S(z)$ for 27 depth profiles.

intercept on the light scatter axis of the plot. PAK *et al.* (1988) presented a plausible theory for interpreting the relationship between chlorophyll (from fluorescence) and phytoplankton biomass (from beam attenuance) based on depth-dependent adaptation to light intensity and nutrient concentration. Our results (Fig. 12) do not include contributions from non-chlorophyllous particles and thus offer support to the theory without the difficulty of partitioning the beam attenuation coefficient to phytoplankton alone.

In summary, the single cell approach is effective and useful at characterising the bulk properties of phytoplankton. To the extent that flow cytometric measurements of forward light scatter and red fluorescence indicate the content of cell carbon and chlorophyll respectively amongst various phytoplankton taxa, our results re-emphasise the important distinction between carbon and chlorophyll as indices of phytoplankton biomass. In the oceanic North Atlantic, maxima in ultraphytoplankton abundance and carbon biomass are located at depths shallower than the maximum in chlorophyll.

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