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Vertical distribution of North Atlantic ultraphytoplankton: analysis by flow cytometry and epifluorescence microscopy

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Abstract—The vertical distribution of numerically abundant ultraphytoplankton at four stations in the central North Atlantic was established using a bench-top mercury-arc lamp flow cytometer and epifluorescence microscopy. Cyanobacteria were identified on the basis of phycoerythrin fluorescence. Red-fluorescing cells (presumably eukaryotic algae) were categorized on the basis of Coulter volume and/or size and number of chloroplasts. The data revealed a diverse community of red fluorescent cell types, including a population of very abundant (but unidentified) cells that frequently were more numerous and slightly smaller than the cyanobacteria. While eukaryotic picoplankton have been reported previously, the overwhelming numerical dominance of these cells in some cases (>90% of total) has not been emphasized.

Essentially all chroococcoid cyanobacteria contained a phycoerythrin composed of phycourobilin and phycoerythrobilin. Average size of the cyanobacteria increased with depth whereas average size of the eukaryotes decreased with depth. Fluorescence per cell increased with depth for all cell types. A five-parameter mathematical equation was introduced to describe the vertical distribution of cell numbers and chlorophyll *a*. This permitted objective estimation of the depths at which cells or chlorophyll *a* reached their maximum (z_{max}) and their zero (z_0) values. In almost all cases, z_0 was greater than the depth of 0.1% surface irradiation and the z_{max} for eukaryotic cells was greater than the z_{max} for cyanobacteria.

INTRODUCTION

ULTRAPLANKTON (including picoplankton) are important members of the marine phytoplankton (KNIGHT-JONES and WALNE, 1951; WOOD and DAVIS, 1956; PLATT and LI, 1986). In recent years, their vertical distribution in the photic zone has been established by several methods of enumeration: light microscopy following serial dilution culture (THRONDSEN, 1969; FURUYA and MARUMO, 1983), epifluorescence microscopy (WATERBURY *et al.*, 1979, 1986; DAVIS *et al.*, 1985; GLOVER *et al.*, 1985a,b, 1986a,b; MURPHY and HAUGEN, 1985; CRAIG, 1986; EL HAG and FOGG, 1986; ZAIKA, 1986; CAMPBELL and CARPENTER, 1987; BOOTH, 1988) and flow cytometry (OLSON *et al.*, 1985, 1988; CHISHOLM *et al.*, 1986).

The use of flow cytometers to detect, enumerate and characterize photosynthetic cells according to pigment autofluoresence began in the late 1970s (HUTTER and EIPEL, 1978; PAAU *et al.*, 1978, 1979; LEGNER and DESORTOVA, 1979) and is now well established (YENTSCH and YENTSCH, 1984; YENTSCH and POMPONI, 1986; BURKILL, 1987). Flow cytometry is well suited to distinguishing cells whose autofluorescence is due mainly to phycoerythrin from those due to chlorophyll *a* because of their widely separated peaks of

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fluorescence emissions (Wood *et al.*, 1985; Yentsch *et al.*, 1986; Li, 1988). Flow cytometry is also well suited to analysing natural populations of autofluorescent marine ultraplankton because they occur at suitable densities. These features have been exploited with success by Olson, Chisholm and coworkers in their shipboard studies of the phycoerythrin-containing cyanobacterial picoplankter *Synechococcus* using an argon laser instrument (Olson *et al.*, 1985, 1988; Chisholm *et al.*, 1986).

We describe here highly resolved depth profiles of cell number, cell size and fluorescence characteristics of numerical abundant autofluorescent cells obtained by arc-lamp flow cytometry and epifluorescence microscopy. Our results indicate that an unidentified group of very small red-fluorescing bodies achieved high densities in many of our deep samples. Due to limitations of the current methodology, we have been restricted to considering the numerically abundant forms although we realise that less abundant phytoplankters may have an ecological role disproportionate to their numbers because of their usually larger size (Goldman, 1988).

METHODS

Sampling

Seawater was collected on CSS *Hudson* cruise 87-022 in the central North Atlantic Ocean during June and July 1987 using a pump sampler system (Herman *et al.*, 1984) for depths less than 110 m and a rosette of Go-Flo bottles for greater depths. Four sites were studied: Sta. Purple (31°57.1′N, 55°36.2′W), Sta. Indigo (34°40.0′N, 54°13.2′W), the Nashville seamount (34°38.9′N, 56°48.3′W) and the Yakutat seamount (34°35.5′N, 50°59.4′W). Each station was sampled on at least 2 or 3 successive days. Water temperature and *in situ* chlorophyll fluorescence were profiled using a CTD (Guildline Model 8705) and submersible fluorometer (Aquatracka). Standard techniques were employed to measure bulk Chl *a* on Whatman GF/F filters (Holm-Hansen *et al.*, 1965) and to enumerate heterotrophic bacteria on 0.2 μm Nuclepore filters (Porter and Feig, 1980).

Flow cytometry

Samples for flow cytometry were analysed on board ship without treatment, except where noted. The common practice of screening samples through a mesh to remove large particles that may clog the orifice proved to be unnecessary in these waters. A FACS Analyser (Becton Dickinson, Mountainview, CA) was operated with a 50 μm orifice and a mercury arc-lamp emitting at 546 nm. Phytoplankton autofluorescence was reflected off a 555 nm dichroic filter and then split into long and short wavelengh components by a 590 nm dichroic filter. The two components were further isolated by passage through a 630 nm long pass absorbance filter (for Chl *a* fluorescence) and a 575 ± 13 nm bandpass filter (for phycoerythrin fluorescence), respectively. Coulter volume also was measured. Both volume and fluorescence were recorded in relative units on three decade logarithmic scales. The instrument was operated without spectral compensation and all particles whose fluorescence beyond 630 nm exceeded the lowest possible instrument threshold were accepted into list mode data acquisition. This made it possible to detect picoplankters that would not have passed the low level discrimination of the volume parameter threshold. From analyses of laboratory cultures (Li, 1988), it has been demonstrated that these optical and electronic configurations allow (i) an unambiguous discrimination between cells that do or do not contain phycoerythrin and (ii) a possible discrimination of different non-phycoerythrin-containing species on the basis of volume and >630 nm fluorescence intensity. Notwithstanding that 546 nm light excites the accessory photopigments rather than Chl *a* itself, LI (1988) has shown that even chlorophytes, which have the lowest chlorophyll accessory pigment ratio ("CAP" of YENTSCH and PHINNEY, 1985) among the common phytoplankton groups emit sufficient red fluorescence from 546 nm excitation to be useful in ataxanomic analyses. Instrument performance, volume calibration and sample flow rate were frequently monitored by analysis of fluorescence calibration beads (PHINNEY *et al.*, 1988a,b; LI, 1986). Depending on the cell concentrations, 0.25–1 ml of sample was analysed.

Epifluorescence microscopy

Duplicate slides were prepared from each sample by filtering 15–20 ml of seawater onto 0.2 μm Nuclepore membranes pre-stained with Irgalin black. Filtered samples were always kept in the dark. They were frozen at –30°C unless counted within an hour during which they were left at room temperature. Most samples were counted using a Zeiss standard microscope equipped with a 50-W mercury lamp, ×100 Neofluor lens and ×15 eyepieces, blue excitation (Zeiss filter combination 487707), green excitation (Zeiss 487712), and both an ocular micrometer grid and ocular linear measuring scale. Occasionally, when the fluorescence intensity of cells was weak, we used a Leitz Orthoplan microscope equipped with a 100-W mercury lamp. Cyanobacteria fluoresced yellow-orange (blue excitation) or orange-red (green excitation). Eukaryotic algae fluoresced deep-red (blue excitation) or not detectably (green excitation).

At least 100–250 cyanobacteria and 50–150 eukaryotes were counted from each filter. All cyanobacteria were checked for the presence of phycourobilin chromophore (WOOD *et al.*, 1985). For selected samples, 40 individual eukaryotic cells were classified according to apparent size and shape of the chloroplasts. Although chloroplast number can vary slightly depending on the conditions of growth (GUILLARD and RYTHER, 1962), chloroplast number and shape are fundamental taxonomic characters for species discrimination (CUPP, 1943; THRONDSEN, 1969). When two chloroplasts of roughly identical shape, size and fluorescence intensity occurred close together, they were assumed to be paired in a single cell. Apparent microscopic size served only to discriminate cell types, not to indicate absolute size. Fluorescence intensity affects apparent cell size and in eukaryotes where only the chloroplast is fluorescent, the actual cell may greatly exceed its chloroplast in size.

Curve fitting

Depth profiles of cell counts and Chl *a* were analysed by the following model which expresses N (cells ml^{-1} or μg Chl *a* l^{-1}) as a function of depth Z (m).

$$N = \left\{\theta\left(1 - \exp\left(-\tau \frac{I^*}{\theta}\right)\right)\left(\exp\left(-\delta \frac{I^*}{\theta}\right)\right)\right\} + N_o,$$

where

$$I^* = I_o\{\exp(-kz) - \exp(-kz_c)\}.$$

This equation contains two constants: I_o(μE m^{-2} s^{-1}), the incident surface irradiance;

and k (m^{-1}), the light attenuance coefficient. It is necessary to estimate the values of five parameters: N_o (cells ml^{-1} or μg Chl *a* l^{-1}), the finite (non-zero) value of N at the water surface; z_c (m), the depth at which $N = 0$ when $N_o = 0$; θ (cells ml^{-1} or μg Chl *a* l^{-1}), a scaling factor; τ (cells ml^{-1} ($\mu E\ m^{-2}\ s^{-1})^{-1}$ or μg Chl *a* l^{-1} ($\mu E\ m^{-2}\ s^{-1})^{-1}$), a parameter related to the change of N with z at low light levels; and δ (same units as τ), a parameter related to the change of N with z at high light levels.

Values of I_o and k were derived from light transmission data (photosynthetically active radiation) measured by a submersible spectroradiometer (Biospherical Instruments MER 1000). Model parameters were estimated by microcomputer using a non-linear curve fitting algorithm (Marquardt, 1963). The depths at which N was maximum (z_{max}) and zero (z_0) were calculated by iteration from the best-fit curves.

RESULTS

Cytogram signature

The general features of the cytograms were similar in most of the samples. Four groups of cells were indicated by their fluorescence and volume signatures. The cyanobacteria were distinguished clearly by phycoerythrin fluorescence (group 1, Fig. 1A) and by their small volume.

Three groups of cells that did not contain phycoerythrin were evident (Fig. 1B). One of these was cells with strong >630 nm fluorescence (group 2, Fig. 1B). The heterogeneity of this group can be seen clearly in its projection onto either the volume or fluorescence frequency distributions (Fig. 1B). We call this group the "large eukaryotes", although many of these cells filtered through 3 μm Nuclepore membranes.

A second group of non-phycoerythrin-containing cells had moderate >630 nm fluorescence. These cells were smaller than the large eukaryotes, as all of them filtered through 2 μm and about 40% through 1 μm (group 3, Fig. 1B). We call this group of picoplankton the "small eukaryotes".

A third group of non-phycoerythrin-containing cells (or particles) had very weak >630 nm fluorescence and was often numerically dominant over other eukaryotic groups and cyanobacteria (group 4, Fig. 1B). These very small red fluorescing bodies (VSRF bodies) filtered through 1 μm but not through 0.4 μm. They also were visible under epifluorescence microscopy as small, faint red spots, but only when slides were freshly prepared and viewed within a few minutes (<*ca.* 15 min) after sample filtration. Furthermore, it was necessary that the slides were epi-illuminated only after the viewer had been completely acclimated to darkness. Due to their weak fluorescence, this group was not always detectable by flow cytometry.

FACS vs microscope counts

There did not appear to be any systematic bias when cyanobacteria were counted by flow cytometry (Fig. 2). However, in many cases (but not all), more eukaryotes were counted by flow cytometry than by microscopy even when VSRF bodies were excluded from comparison (Fig. 2). In principle, the presence of chlorophyll-containing detritus would render an overestimation in the flow cytometric counts. However, since microscopic examination showed that such material was insufficient to account for the difference, we suspected that a delay in the microscopic counting may have resulted in an underestimation of the eukaryotic (but not the cyanobacterial cell) numbers. We tested

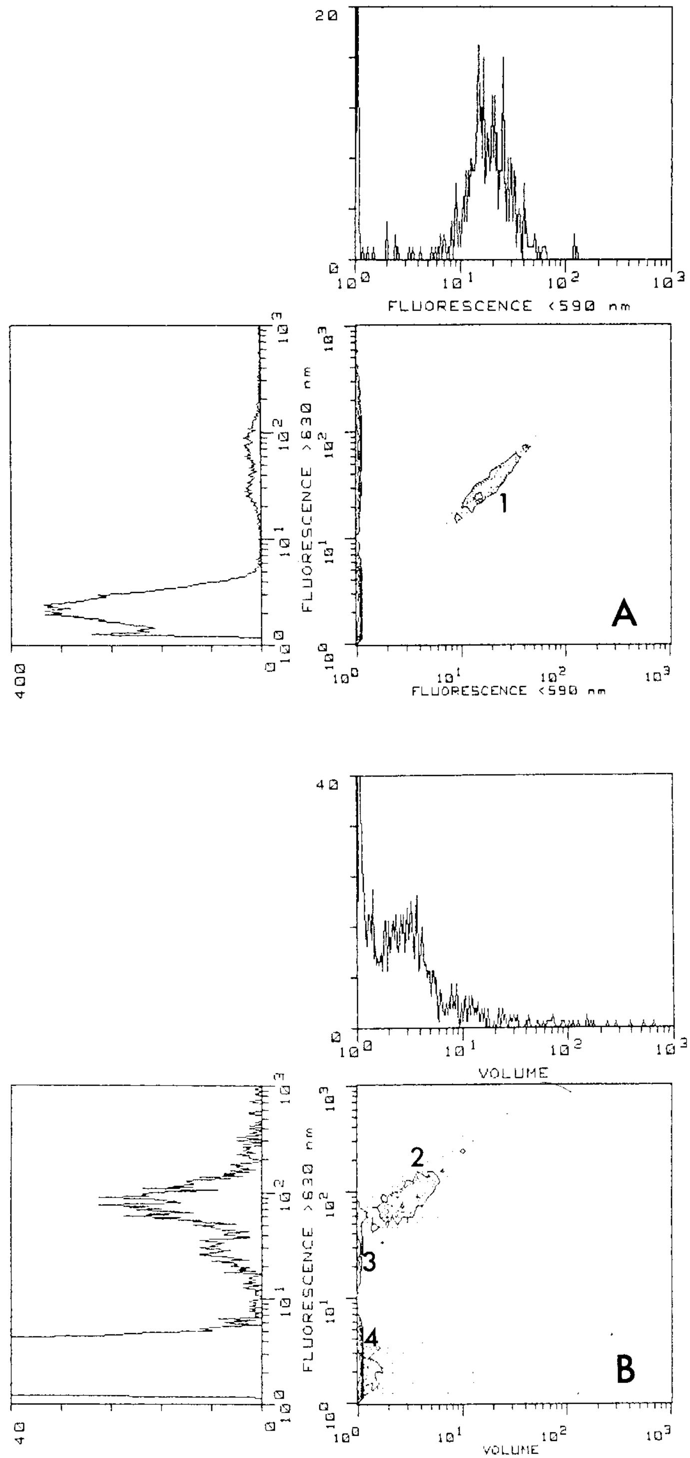

Fig. 1. Two-dimensional contour plots (cytograms) and projected frequency distributions of a typical central North Atlantic plankton sample showing separation based on (A) $F > 630$ nm vs $F < 590$ nm and (B) $F > 630$ nm vs Coulter volume. See text for a discussion of the four groups.

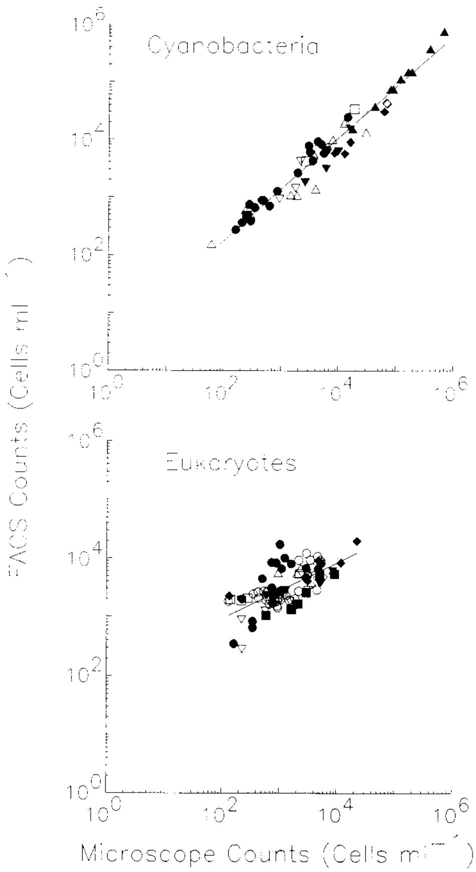

Fig. 2. Comparison of cell counts obtained by flow cytometry and epifluorescence microscopy. For eukaryotes, all cells <20 μm from microscopy were compared against the sum of groups 2 ("large eukaryotes") and 3 ("small eukaryotes") from cytometry. Different symbols represent different stations. Solid triangles for cyanobacteria refer to a laboratory culture of *Synechococcus* WH7803 ($n = 11$). Solid line is $\log(Y) = a + b \log(X)$; for cyanobacteria, $a = 0.46$, $b = 0.89$, $r^2 = 0.95$, $n = 58$; for eukaryotes, $a = 0.20$, $b = 0.48$, $r^2 = 0.46$, $n = 95$. Faint dotted line has a slope of 1.

this by comparing slide preparations counted immediately (about 5 min) after sampling against replicates counted after the normal protocol (counted within an hour of filtration or frozen immediately after filtration and counted within a few days, usually within a few hours). In three trials, only 44 ± 28% of the freshly counted eukaryotes were accounted for in the normal protocol. This contrasted with an accounting of 108 ± 24% of the cyanobacteria.

Depth profiles

Water structure. The depth profiles of temperature and σ_t were essentially mirror images of each other (Fig. 3). Each station was characterized by a rather extensive pcynocline. Station Purple differed in that the base of its pcynocline was at least a few tens of meters deeper than those of the other stations.

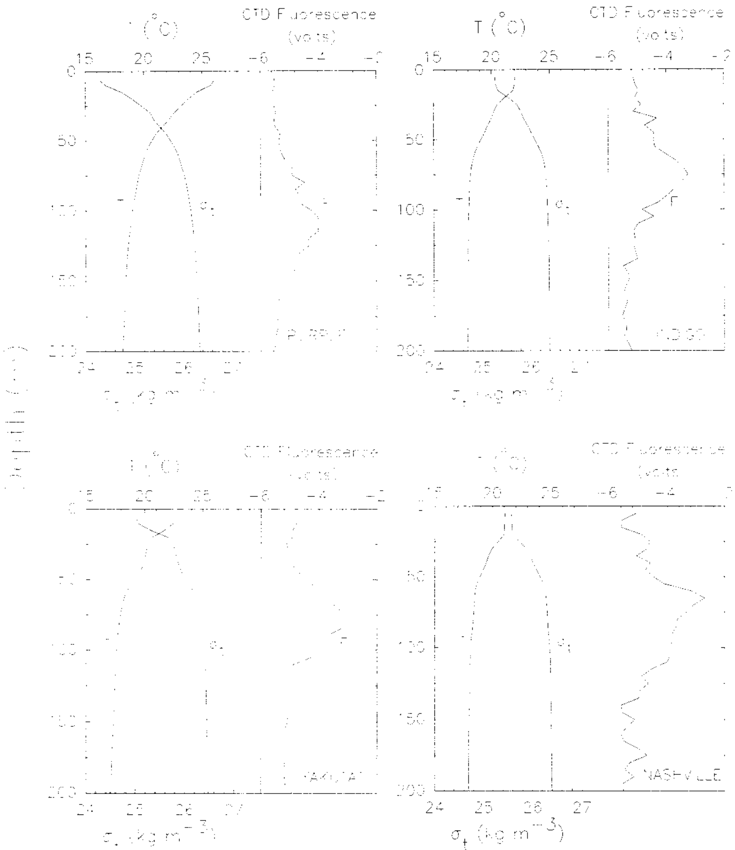

Fig. 3. Depth profiles of water temperature, σ_t and relative *in situ* fluorescence as measured by CTD and submersible fluorometer.

Chlorophyll a *and bacteria.* Relative *in situ* fluorescence from the profiling submersible fluorometer indicated a well-developed subsurface maximum at each station (Fig. 3) which was confirmed by measurements of extracted Chl *a* in discrete samples (Fig. 4). The $N(z)$ model generally gave good fits to the Chl *a* data, in particular its representation of the asymmetry about the maximum—reaching a value of N_o at the surface and zero at a depth below the maximum. Figure 5 shows values of z_{max} and z_0 calculated from the $N(z)$ model, and the $\%I_{surface}$ to which these two depths correspond. Station Purple differed from the other three more northerly stations in having the deepest (112 m; 0.5% $I_{surface}$) and lowest amplitude (0.31 μg l^{-1}) maximum. The base of the chlorophyll distribution (z_0) did not exceed 0.1% $I_{surface}$ at any station (Fig. 5).

The subsurface bacterial peaks were less well developed (Fig. 4). According to the model, bacterial z_{max} was about the same (48–55 m) at all stations (Fig. 5).

Cyanobacteria. At all stations, the mean phycoerythrin fluorescence per cyanobacterium measured by FACS increased with depth (Fig. 6). The modal fluorescence per cell increased in a similar manner (not shown). Above 60–80 m, depending on the station, the FACS phycoerythrin fluorescence was undetectable. We relied solely on epifluorescence microscopy to count cyanobacteria at these upper depths. Cyanobacterial density

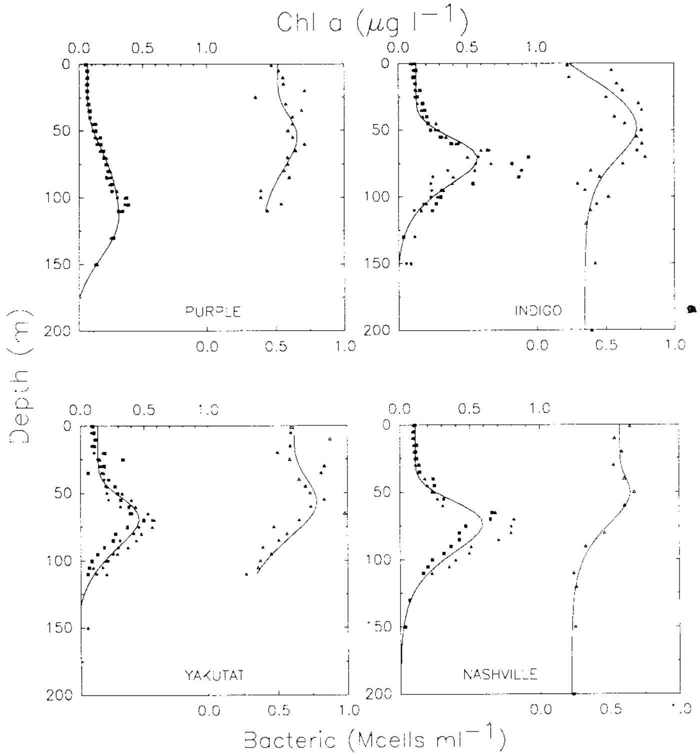

Fig. 4. Depth profiles of chlorophyll *a* (left half of each panel, upper *x*-axis label) and heterotrophic bacteria (right half of each panel, lower *x*-axis label). Different symbols represent different days on the same station. Solid curves are best fits of the $N(z)$ model.

was fairly uniform at shallow depths so that the subsurface maximum was poorly developed (Fig. 6). Nevertheless, the $N(z)$ model gave estimates of z_{max} ranging from 27 to 48 m for the four stations, considerably less than the corresponding z_{max} for Chl *a* (Fig. 5).

Wood *et al.* (1985) showed that a switch from green to blue light excitation had little effect on the apparent fluorescence of cyanobacteria that contain a phycoerythrin composed of phycourobilin (PUB) and phycoerythrobilin (PEB) chromophores. However, such a switch substantially decreased the autofluorescence of cells containing only PEB chromophores. On the basis of this technique, all cyanobacteria observed in the present study were deemed to have both chromophores.

We also noted that large cyanobacteria (retained on 1 or 3 μm filters) comprised a greater proportion of the population at depth and that, above 70 m, most of the cyanobacteria were small (passed 1 μm filter) (Fig. 7). From microscopy, we found that surface populations were more heterogeneous than deeper ones in both size and fluorescence intensity. Cells near the surface were often rod-shaped whereas deeper cells were usually coccoid. Although the average size of cyanobacteria increased with depth, the very largest cells were seen near the surface. These large cyanobacteria were rod-shaped of normal diameter but as much as 5 μm in length.

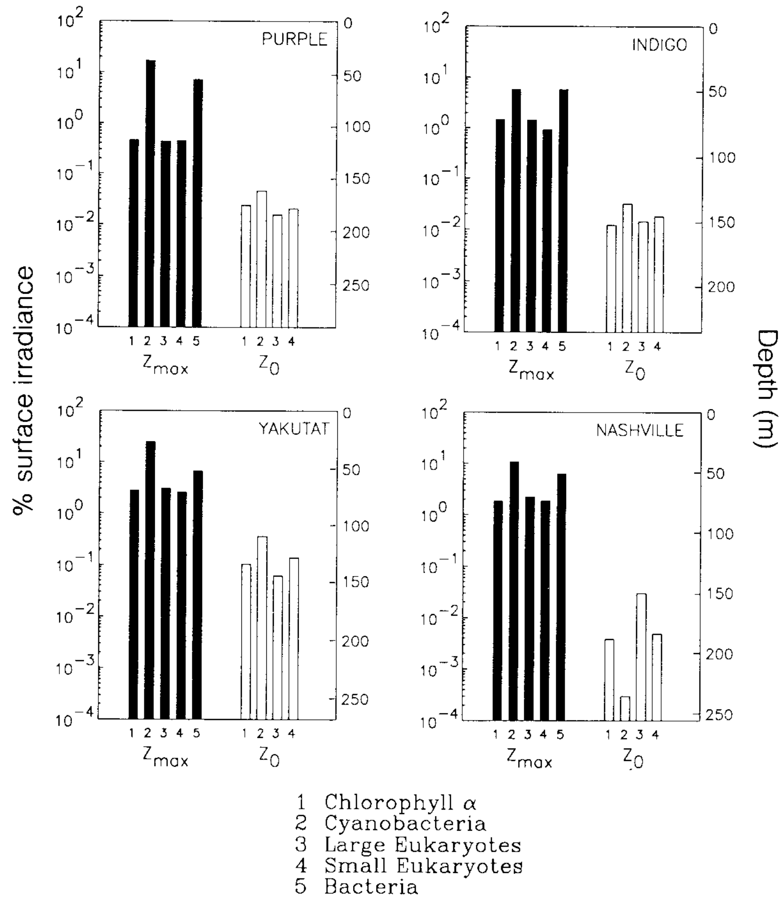

Fig. 5. Depths (right y-axis label) and corresponding percent surface irradiance (left y-axis label) at which chlorophyll a, cyanobacteria, large eukaryotes, small eukaryotes and bacteria at the four stations reach their maximum (z_{max}) and zero (z_0) as calculated from the best fitting $N(z)$ model.

Large and small eukaryotes. At all stations, the mean Chl a fluorescence per cell measured by FACS increased with depth (Figs 8 and 9). The modal fluorescence per cell increased in a similar manner (not shown). Unlike the cyanobacteria, both large and small eukaryotes were detectable by FACS, even at the lowest levels of fluorescence occurring at the shallowest depths. A subsurface maximum in eukaryote density was clearly evident at all stations and, in each case, the z_{max} for eukaryotic cell density did not differ from that for Chl a (Fig. 5). There was a slight hint of a local maximum in mean fluorescence per cell at z_{max} (Figs 8 and 9). The lower limit of the occurrence of eukaryote cells (z_0) was similar to the lower limit for detectable Chl a at each station (Fig. 5).

In contrast to the cyanobacteria, the eukaryote assemblages at shallower depths had a greater proportion of large cells. For example, in the "large eukaryote" group defined by flow cytometry, the modal cell diameter changed only slightly (average modal diameter = 2.2 μm) throughout the water column (Fig. 10), suggesting that the major assemb-

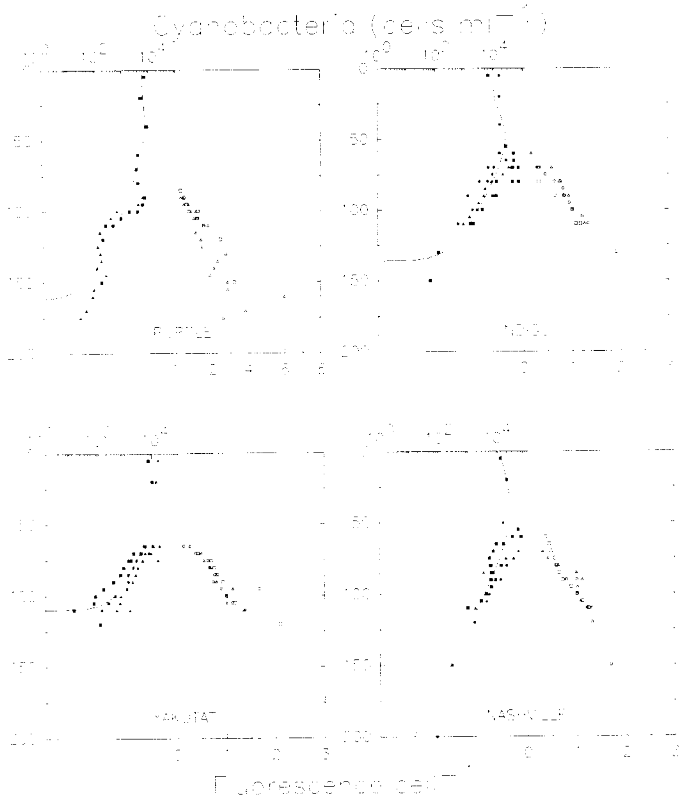

Fig. 6. Depth profiles of cyanobacteria cell density (solid symbols, left half of each panel, upper x-axis label) and relative mean phycoerythrin fluorescence per cell (open symbols, right half of each panel, lower x-axis label). Relative mean fluorescence per cell at depth z is the mean fluorescence at depth z divided by the mean fluorescence at 100 m. Different symbols represent different days on the same station. Solid curve is best fit of the $N(z)$ model.

lage component remained the same. However, the mean cell diameter decreased substantially with depth (Fig. 10), indicating that the shallower samples contained a relatively greater number of larger cells.

We evaluated the taxonomic complexity of eukaryotic cells by placing them into numerous categories defined by size and shape of the fluorescent regions (Figs 11 and 12). Two major categories of red-fluorescent particles were defined by this classification. Those of distinct shape and even margins, and those of irregular shape and indistinct margins. We believe the latter to be cells whose chloroplasts were sufficiently convoluted that an irregular face was presented to the microscope; we rule out the probability that they were detritus because they did not show the "off-colour" fluorescence (spots of blue, white or yellow) we commonly see in detritus.

Among the chloroplasts that were regularly shaped, there were six recurrent groups: single round chloroplasts of apparent diameters <2 μm, 2–3 μm or 3–4 μm; and paired chloroplasts in all three of the size categories. We do not know if the paired chloroplasts were truly two distinct plastids such as occur in *Chrysochromulina minor* (Manton and Leedale, 1961) or if they were distal ends of a large chloroplast which extended around

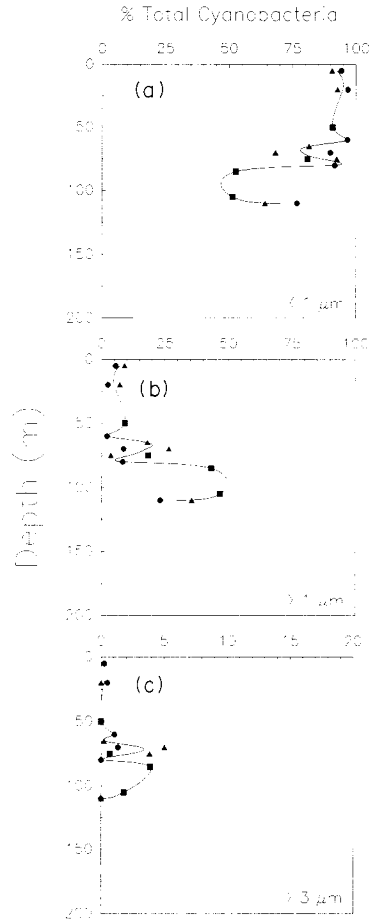

Fig. 7. Depth variation of the percentage of total cyanobacteria that passed 1 μm membranes (a), that was retained on 1 μm membranes (b), and that was retained on 3 μm membranes (c). Station Purple (circles), Sta. Yakutat (triangles), Sta. Nashville (squares). Curves are cubic spline interpolation through mean values at each depth.

three sides of the cell (cf. *Ochromonas danica*, Fig. 6 in Gibbs, 1962), but their orientation and similar size and shape were highly suggestive of their occurrence inside a single cell.

The detailed microscopic examinations of red-fluorescing cells indicated that small cells were relatively more abundant at depth than near the surface (Figs 11 and 12), in agreement with the FACS volume measurements (Fig. 10). The cumulative size distributations at Sta. Yakutat show that although cells >4 μm comprised a significant percentage of the total eukaryotes at 20 and 65 m, these cells were almost all absent at 70 and 80 m (Fig. 11). The median of the size distribution shifted from the 3–4 μm category at 20 and 65 m to the 2–3 μm category at 70 and 80 m (Fig. 11). At Sta. Purple, almost all the eukaryotes in the shallow samples (20 and 40 m) were 3–4 μm or greater (Fig. 12); there may be some uncertainty in this particular observation because of the very small number of cells on which the percentages were based (only 8 at 20 m and 7 at 40 m).

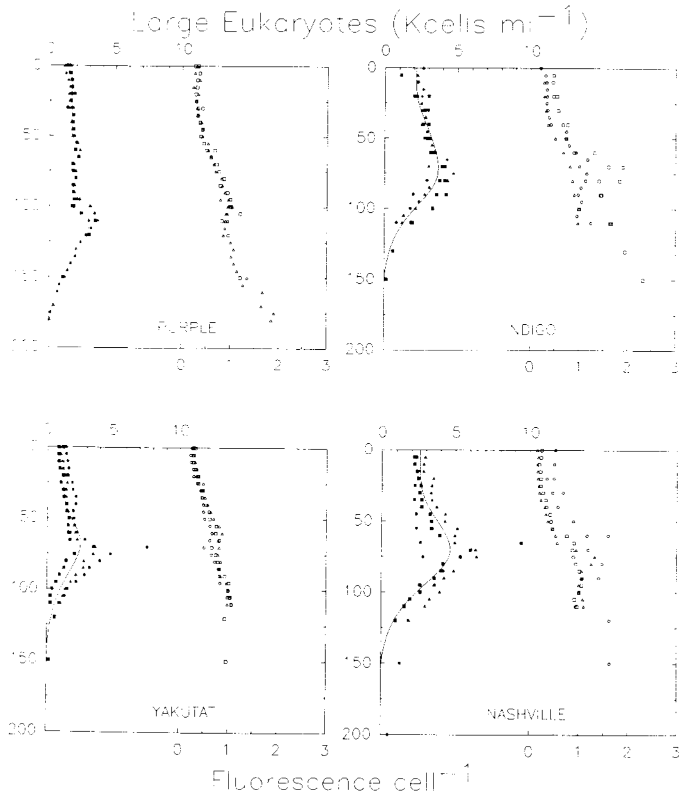

Fig. 8. Same as Fig. 6 except for large eukaryotes and relative mean chlorophyll *a* fluorescence per cell.

Although at this station the deeper samples also contained >4 μm cells, the median of the size distribution followed the same depth-related trend as at Sta. Yakutat; namely a decrease with depth (Fig. 12).

Epifluorescence microscopy rarely revealed particles that could be interpreted as phytoplankton cells with numerous chloroplasts. We occasionally observed particles that seemed to have several cyanobacteria and one or two red-fluorescing chloroplasts. Although these may have been phagotrophic protozoa, they were included in Figs 11 and 12. Using transmitted light to examine material retained on 3 μm filters, we estimated that about 5% of the single cyanobacteria and/or the <4 μm eukaryotes appeared to be inside large non-fluorescent particles.

VSRF Bodies. Mean Chl *a* fluorescence per body increased with depth (Fig. 13). Due to their extremely weak fluorescence, these bodies were undetectable by FACS above 125 m at Sta. Purple and above 85 m at Sta. Yakutat (Fig. 13).

Where detected, VSRF bodies greatly outnumbered the sum of cyanobacteria, large and small eukaryotes. For example, at Sta. Purple where we had counts of all four groups at depths ⩾125 m, VSRF bodies comprised 68% (at 175 m) to 94% (at 125 m) of the total number of autofluorescent particles (Fig. 14). To assess the distribution of biomass among these groups, we converted cell numbers to carbon content as follows. For the

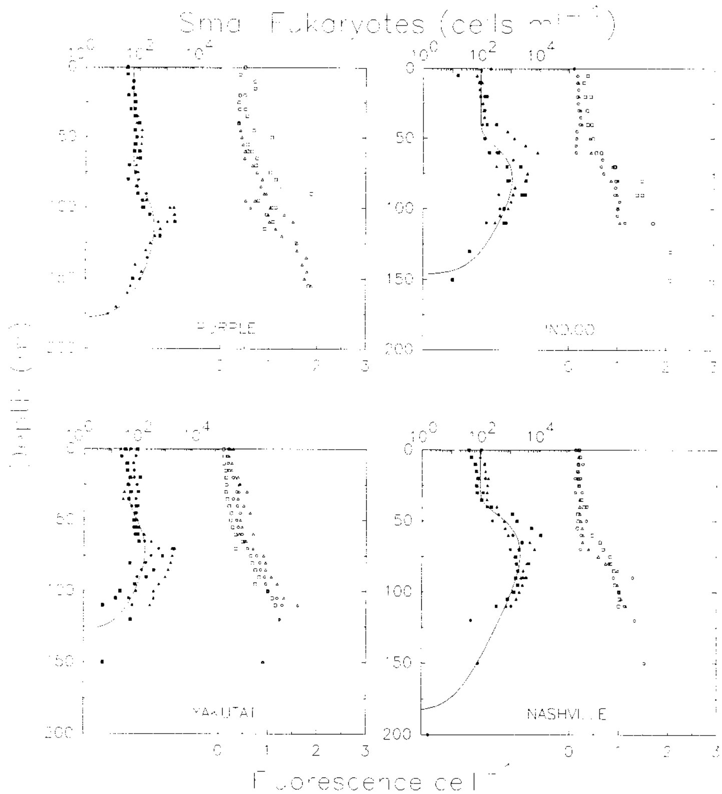

Fig. 9. Same as Fig. 6 except for small eukaryotes and relative mean chlorophyll *a* fluorescence per cell.

large eukaryotes, carbon cell^{-1} was based on STRATHMANN'S (1967) carbon:volume conversion using the measured Coulter volume spectra. For the small eukaryotes and VSRF bodies, we assumed diameters of 1 and 0.7 μm, respectively, and used 220 fg μm^3 for conversion to carbon as suggested by BOOTH (1988). For cyanobacteria, we also followed BOOTH (1988) in using CUHEL and WATERBURY'S (1984) value of 294 fg cell^{-1} derived from nutrient-sufficient laboratory cultures. This seemed appropriate because we were considering the larger cyanobacteria deep in the water column (≥125 m) (cf. Fig. 7) where nutrients would have been sufficient. Furthermore, as KANA and GLIBERT (1987) showed, the carbon content of *Synechococcus* is invariant at about 250 fg cell^{-1} over a wide range of growth irradiance. At 130 m, these calculations give the following carbon biomass for cyanobacteria, small and large eukaryotes, and VSRF bodies, respectively: 0.045, 0.020, 5.6 and 0.51 μg l^{-1}. The sum of these is 6.2 μg C l^{-1}. In comparison, the measured Chl *a* biomass at this depth was 0.27 μg l^{-1}. This converts to 6.1 μg C l^{-1} using a C:Chl ratio of 22.5 which is appropriate for nutrient-sufficient algae at the prevailing temperature and irradiance (GEIDER, 1987). We can therefore account for all the measured Chl *a* based on cell counts. At depths below 125 m at Sta. Purple, the large eukaryotes accounted for more than 90% of the carbon biomass. VSRF bodies made up most of the balance while the contributions from cyanobacteria and small eukaryotes were very small (Fig. 14).

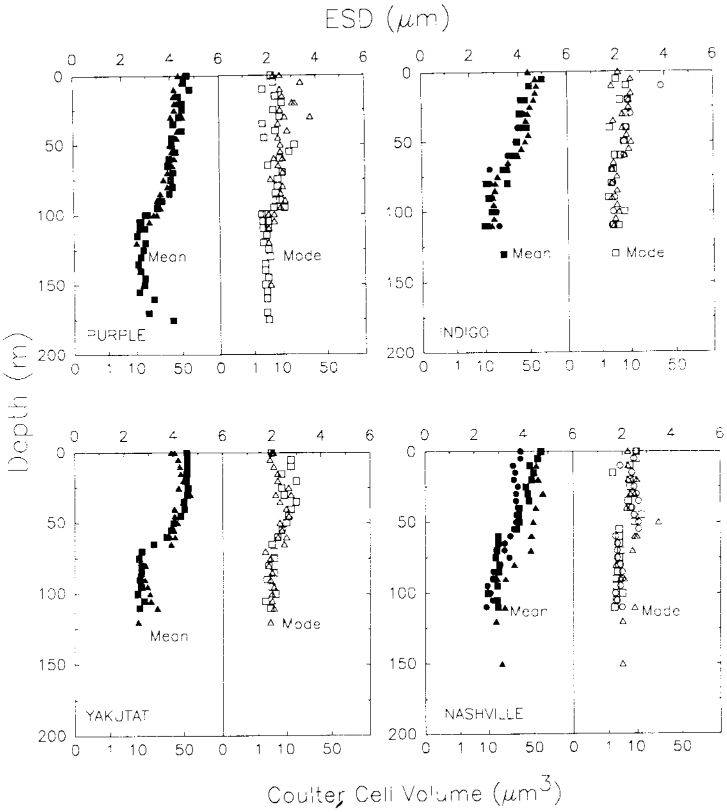

Fig. 10. Depth variation of equivalent spherical diameter (ESD) of the "large eukaryotes" as defined by flow cytometry (group 2, Fig. 1B). The mean diameter is represented by the solid symbols in the left half of each panel; the modal diameter is represented by the open symbols in the right half of each panel. Different symbols represent different days on the same station.

DISCUSSION

A major aim of our work was to evaluate the utility of the FACS Analyser in shipboard research on open ocean ultraphytoplankton. In our hands, the instrument failed to detect weakly fluorescing cyanobacteria and VSRF bodies in shallow samples; it also failed to give a Coulter volume measurement for all but the so-called large eukaryotes. In spite of these failures, and given the inherent limitations of non-sorting flow cytometry (Phinney *et al.*, 1988a,b), we conclude that the FACS is a useful instrument for allometric and ataxonomic analyses (*sensu* Yentsch *et al.*, 1986; Phinney *et al.*, 1988b) of numerically abundant ultraplankton at sea. This is particularly so because of the instrument's sensitivity to red-fluorescing particles difficult to detect by microscopy and because of the speed at which samples could be processed.

For cyanobacteria, the FACS performed well as a cell counter (Fig. 2). The distinctive fluorescence due to phycoerythrin made it easy to identify and count these cells by either method. On the other hand, the eukaryotes were a diverse assemblage of cells differing in both size and relative fluorescence. The comparison between the number of eukaryotes

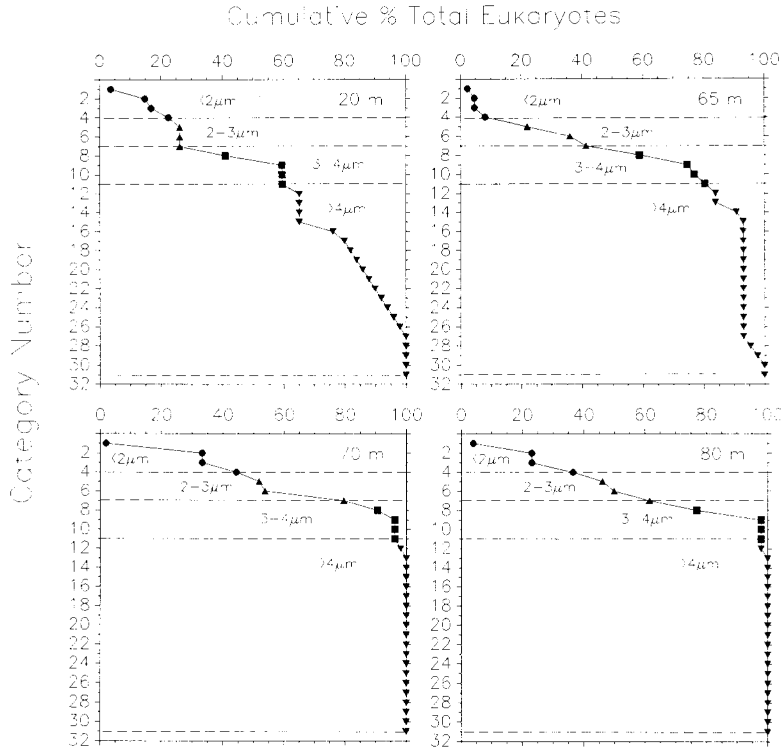

Fig. 11. Cumulative size distribution of microscopically identified eukaryotes at various depths at Sta. Yakutat. In numerical order, the cell categories are as follows: <2 μm paired, <2 μm round, <2 μm with cyanobacteria, <2 μm irregular, 2–3 μm round, 2–3 μm paired, 2–3 μm irregular, 3–4 μm irregular, 3–4 μm paired, 3–4 μm round, 3–4 μm apparently round cell with 3–4 chloroplasts, 4–5 μm irregular, 4–6 μm paired, 4–5 μm square with 4 chloroplasts, 4 × 6 μm round, 6 μm irregular, 8 μm dinoflagellate, 2 × 5 μm paired, >10 μm irregular, 8 μm irregular, 8 μm paired, 4 × 4 μm cell with <2 μm chloroplast, 12 μm round, 10 × 12 μm pointed, 20 μm irregular, >20 μm cell with many irregular chloroplasts, 6 μm round cell with 6 <1 μm chloroplasts, 23 μm round, 20 μm square, 3 × 20 × 10 μm triangle, 14 μm pennate diatom. Unless otherwise noted, dimensions apply to fluorescent portion of the cell.

(excluding VSRF bodies) counted by the two methods (Fig. 2) suffered because the ataxonomic-allometric analysis (Fig. 1B) resulted in cytogram clusters that could not be matched in detail against microscopically determined cell categories (Figs 11 and 12). In addition, there was a significant underestimate by microscopy of the number of red-fluorescing cells when these were not counted within a short time after sampling. The standard techniques of handling samples for microscopy can result in an apparent loss of eukaryotic cells. Flow cytometry, by providing rapid sample processing and sensitive photodetection, can give improved estimates of eukaryotic cell abundance.

Using the epifluorescence technique of Wood *et al.* (1985), we deemed all the observed cyanobacteria to have both PUB and PEB chromophores. This technique is not sensitive enough to distinguish unequivocally between PUB-containing cells that differ in the ratio of PEB:PUB. However we noticed that many of our cells were more easily detected with blue than with green light. This is different from what is observed in Type I ("DC-2") cells (see Wood *et al.*, 1985), where blue and green light of equivalent intensity

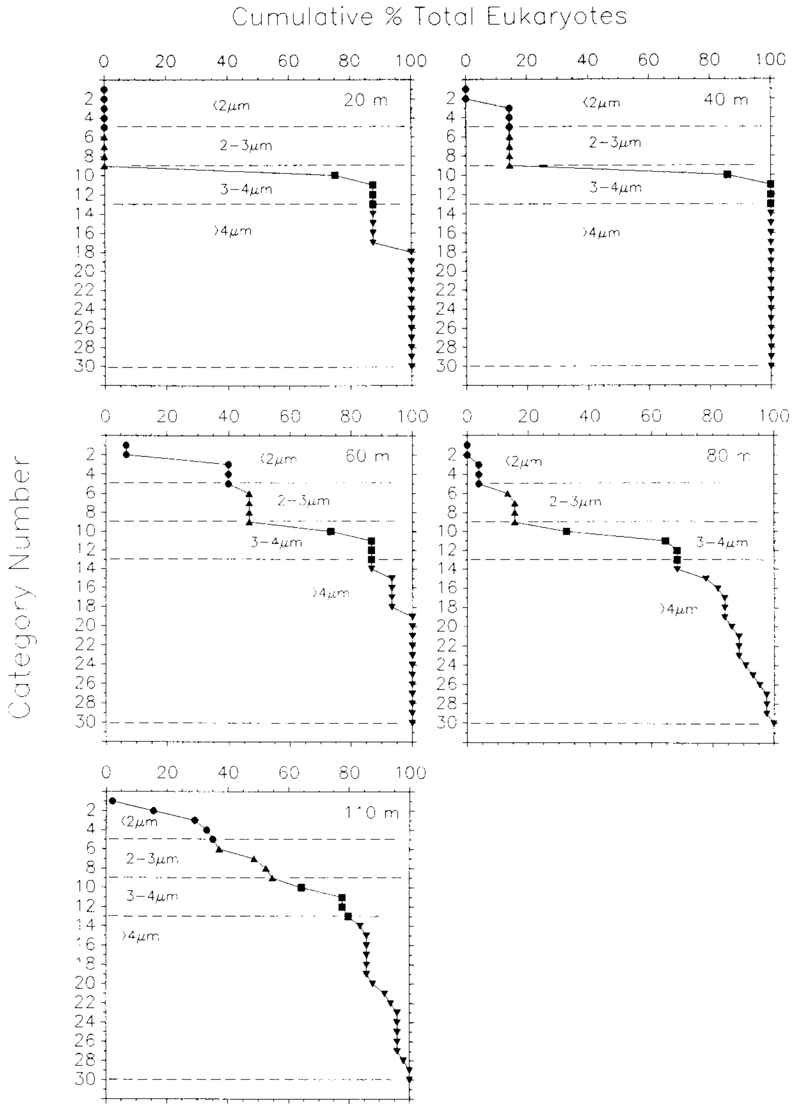

Fig. 12. Cumulative size distribution of microscopically identified eukaryotes at various depths at Sta. Purple. In numerical order, the cell categories are as follows: <2 µm paired, ≤1 µm round, <2 µm round, <2 µm irregular, ≤1 µm irregular, 2–3 µm round, 2–3 µm paired, 2–3 µm irregular, 1 × 2.5 µm crescent, 3–4 µm irregular, 3–4 µm paired, 3–4 µm rectangular, 1 × 3.5 µm irregular, 4–5 µm irregular, 4–6 µm paired, 3 × 6 µm irregular, 3 × 6 µm with 3 star-shaped chloroplasts, 7 × 1 µm irregular, 10 µm paired, 8 µm irregular, 8 µm paired, 8 µm with 1 round chloroplast, 8 µm with many 1 µm chloroplasts, 8 µm apparently rectangular cell with chloroplasts in corner, 16 × 3 µm thin curled chloroplast, 17 µm square with irregular chloroplast, 14 µm irregular cell with chloroplasts in corners, 18 µm round, 16–18 µm irregular, >20 µm cell with many irregular chloroplasts. Unless otherwise noted, dimensions apply to fluorescent portion of the cell.

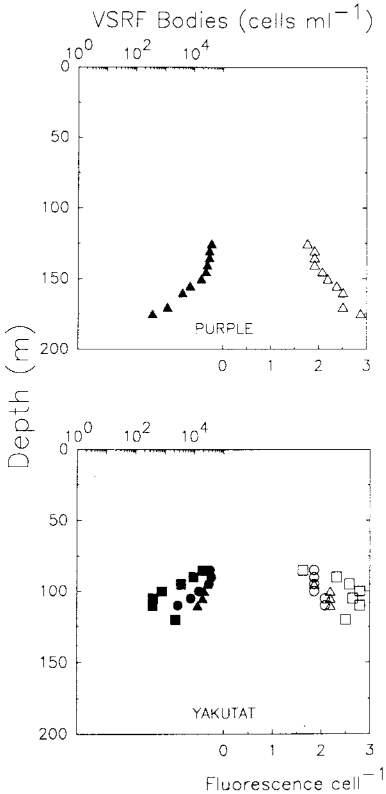

Fig. 13. Same as Fig. 6 except for very small red-fluorescing bodies, and fluorescence per cell at depth z is not expressed relative to fluorescence at 100 m.

cause cells to fluoresce at similar levels. Our results are therefore qualitatively consistent with the report by OLSON *et al.* (1988) that cells with the high PUB phycoerythrin described by ONG *et al.* (1984) are the common forms in the North Atlantic.

We noted that larger cyanobacteria made up a greater proportion of the population at depth (Fig. 7): this has been reported before (GLOVER *et al.*, 1985b, 1986a; CRAIG, 1986). It is consistent with the finding that *Synechococcus* cells grown at low irradiances are larger than those grown at high irradiances (KANA and GLIBERT, 1987). Elongated cyanobacterial cells (up to 5 μm) similar to those in the shallowest samples also occur in cultures, particularly after the onset of lag phase (WOOD, unpublished data). It is possible that this apparent elongation without cytokinesis is a result of extreme high light or nutrient stress. GLIBERT *et al.* (1986) showed that nitrogen-deprived *Synechococcus* WH7803 did not divide but showed indications of cell enlargement.

Information about the vertical distribution of phytoplankton in the photic zone is important in many areas of plankton research. Since Chl *a* is such a widely used index of phytoplankton biomass, depth profiles of this measurement have received much attention (VENRICK *et al.*, 1973; KARABASHEV and SOLOV'YEV, 1978; DANDONNEAU, 1979; CULLEN, 1982; HERBLAND *et al.*, 1985). Recent interest in predicting primary production from chlorophyll-light models (HERMAN and PLATT, 1986) or from remote sensing

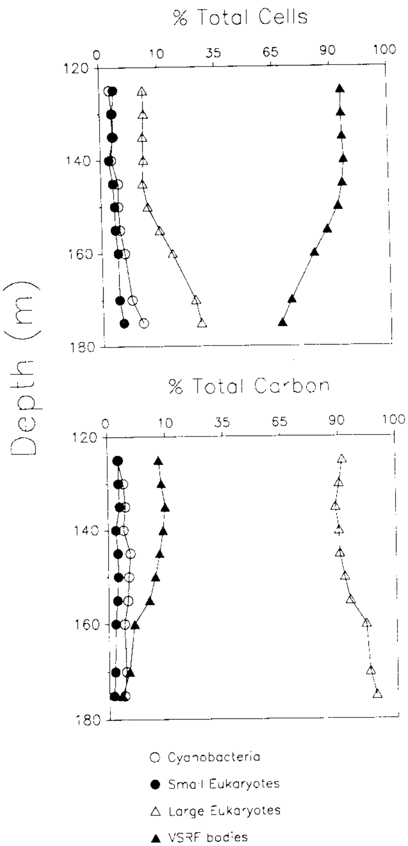

Fig. 14. Percentage composition of total autofluorescent particles below 125 m at Sta. Purple. Top panel shows composition by numbers; bottom panel shows composition by carbon content calculated as described in the text. Abscissa values are expressed in the angular transformation scale (arcsin √p) which is appropriate for percentages (Sokal and Rohlf, 1969).

algorithms (Platt, 1986; Platt and Lewis, 1987; Campbell and O'Reilly, 1988) points out the need for a general mathematical description of the variation of Chl *a* with depth. Platt *et al.* (1988) suggested using a Gaussian curve modified by the additive inclusion of a term to account for the baseline chlorophyll level.

In describing the depth profiles of Chl *a* and the various cell types, we found the modified Gaussian curve of Platt *et al.* (1988) to be unsuitable for the following reasons. First, the modified Gaussian does not reproduce the asymmetry about the maximum observed in many profiles. Second, the modified Gaussian never attains a value of zero and therefore does not estimate the depth at which Chl *a* or cell density becomes undetectable. Our $N(z)$ model overcomes both these shortcomings, but at the expense of an additional parameter. Although we have not done so, it would be simple to evaluate the integral of $N(z)$ from the sea surface to z_0 to obtain water column biomass. Furthermore, the $N(z)$ model could be used to calculated depth-profile descriptors such as "hardness" (Dandonneau, 1979) and "vertical structure index" (Cullen and Eppley, 1981; Napp, 1987).

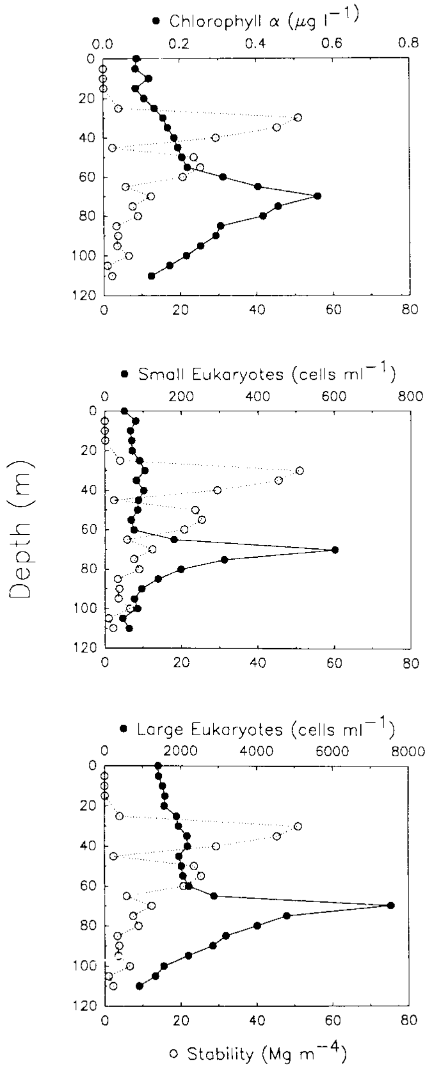

Fig. 15. Depth profiles of chlorophyll *a*, small eukaryotes, large eukaryotes, and vertical stability ($d\sigma_t dz^{-1}$) at Sta. Yakutat on 27 June 1987.

One notable feature in some of our depth profiles was a very sharp subsurface maximum. For example, at Sta. Yakutat on 27 June, Chl *a*, and especially the number of small and large eukaryotes were very much greater at 70 m than at 65 and 75 m (Fig. 15). The 5 m band centered at 70 m accounted for 11, 21 and 14% of the column-integrated Chl *a*, small and large eukaryotes, respectively. This result emphasizes the importance of collecting samples at close intervals. The mechanism for maintaining such sharp peaks in the face of relatively low water stability ($d\sigma_t dz^{-1}$) (Fig. 15) must be intriguing. The $N(z)$ model poorly represented depth profiles with sharp maxima such as these.

Flow cytometry allowed cell density profiles to be resolved at close intervals through the photic zone; the $N(z)$ model allowed objective and precise estimation of z_{max} and z_0.

From these, there was an unequivocal demonstration of the correspondence between z_{max} of Chl *a* and that of the eukaryotic ultraplankton (Fig. 5). By the same token, z_{max} of cyanobacteria was significantly shallower (Fig. 5). This pattern is general (Glover *et al.*, 1985a,b, 1986a,b; Murphy and Haugen, 1985; Zaika, 1986; Booth, 1988) and reflects the superiority of eukaryotic ultraplankton over cyanobacteria in utilizing the deeply penetrating dim blue-violet light for growth and photosynthesis (Wood, 1985; Glover *et al.*, 1986b, 1987). Significantly, bacterial z_{max} was shallower than chlorophyll z_{max} (Fig. 5). This has been noted before (Ducklow, 1984) and may indicate the association of bacteria with the primary production maximum which is shallower than chlorophyll z_{max} (Irwin and Platt, unpublished data). The maximum depth at which cells were found (z_0) was almost without exception at a level <0.1% of surface irradiance (Fig. 5). We did not measure the photosynthetic performance of phytoplankton at those depths, but Richardson *et al.* (1983) pointed out that the photosynthetic compensation light levels of some algae would be at those depths.

One of the most consistent features in our data was the increase of mean relative fluorescence per cell with depth. In the case of cyanobacteria, the change in mean relative phycoerythrin fluorescence may reflect a change in the relative proportion of "bright" and "dim" subpopulations (Olson *et al.*, 1985; Chisholm *et al.*, 1986). Using 488 nm excitation, Olson *et al.* (1988) identified the "bright" and "dim" subpopulations to be cyanobacteria having high and low PUB chromophore contents, respectively, suggesting a genetic distinction between the subpopulations. Oceanic cyanobacteria also have been characterized by immunofluorescence and found to belong to more than one serogroup (i.e. clusters of strains labeled by one antiserum). Campbell and Carpenter (1987) reported depth-related variations in the percentage of *Synechococcus* that was represented by the WH7803 (warm, oceanic, low-PUB clone) serogroup. Thus, depth changes in relative phycoerythrin fluorescence per cyanobacterium could have been due to changes in the proportion of different genetic or sero-groups, or due to changes in cellular responses to light (Barlow and Alberte, 1985) and nitrogen availability (Glibert *et al.*, 1986), or both.

In the eukaryotic algae, the change in mean Chl *a* fluorescence per cell may also be a cellular response to changing light intensity (Yentsch *et al.*, 1985) or reflects a change in the proportion of "bright" and "dim" subpopulations. Sakshaug *et al.* (1987) showed that a decrease in mean Chl *a* fluorescence per cell in a low- to high-light shifted population (*Thalassiosira pseudonana*) resulted from changing proportions of low and high fluorescing subpopulations, each of which maintained its respective fluorescence level throughout the shift. We believe that in our case, changes in mean Chl *a* fluorescence per cell were a cellular response (photoadaptation) and not a shift in relative proportions of subpopulations. For example, in the large eukaryotes, changes in the mean and modal fluorescence were parallel but changes in the mean and modal cell diameters were not (Fig. 10). The relatively invariant modal cell diameter suggests that the same group of cells formed the major subpopulation throughout the photic zone; at the same time, the increase in modal fluorescence per cell with depth suggests that there was a photoadaptive increase in relative fluorescence per cell within this major subpopulation. This is consistent with observations that under low light conditions, the excitation of Chl *a* fluorescence by accessory pigments increases relative to the excitation by Chl *a* itself (Neori *et al.*, 1984; Soohoo *et al.*, 1986).

The so-called VSRF bodies are interesting because of their numerical dominance (up to 4×10^4 cells ml^{-1}), at least up to the depths where we were able to quantify them (Figs 13 and 14). We have no basis to judge their identity except that they passed through 1 μmfilters (100 mm Hg vacuum pressure) and could be collected on 0.2 μm filters. We cannot rule out the possibility that they may have been free-floating plastids (GIESKES and ELBRÄCHTER, 1986). However, it is known that very small eukaryotic phytoplankton are common (JOHNSON and SIEBURTH, 1982; JOINT and PIPE, 1984; TAKAHASHI and HORI, 1984). In fact, MURPHY and HAUGEN (1985) noted that 63% of oceanic eukaryotes passed 0.8 μm filters and these included representatives of the prasinophytes, prymnesiophytes and chrysophytes. They also noted that many of these cells show little indication of accessory photopigments from their fluorescence excitation spectra. If this were the case for VSRF bodies, it would be consistent with the weak fluorescence we measured under 546 nm excitation. It is possible that VSRF bodies do not correspond to any of the cells recorded by MURPHY and HAUGEN (1985) because in general, the *total* number of their oceanic eukaryotes is about an order of magnitude less than our counts of VSRF bodies at corresponding depths. We suspect that VSRF bodies previously have been unrecognized because of difficulties in detection by epifluorescence microscopy. It is probable that VSRF bodies correspond to the novel free-living prochlorophyte described by CHISHOLM *et al.* (1987, 1988) which they detected by argon-laser flow cytometry. Clearly, further work is necessary to establish their identity, to properly delineate their distribution, abundance and biomass, and to determine their role in marine productivity.

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