Marine Biology 97, 275–286 (1988)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Size classes and major taxonomic groups of phytoplankton at two locations in the subarctic Pacific Ocean in May and August, 1984*

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Abstract

In order to assess the relative importance of the pico- and nanoplankton fractions, the composition of entire phytoplankton communities at Weathership Station P (50°N; 145°W) and at 53°N; 145°W were studied in May and August, 1984, using epifluorescence, scanning electron, and inverted light microscopy. The biomass of major taxa within five size classes was estimated from cell volume and cell concentration. For both months, approximately two-thirds of the total phytoplankton carbon were contributed by cells $< 5\ \mu m$. In May, 16% of plant biomass was contributed by cells $< 2\ \mu m$, and in August 39%. (In both months 90% of plant carbon $< 2\ \mu m$ was contributed by the blue-green coccoid *Synechococcus* spp.) Cells 2 to 5 μm contributed about 39% to total plant carbon; they were mostly flagellates in May and nonmotile coccoids in August. The remaining one-third of algal carbon was composed of dinoflagellates, cryptomonads, other flagellates and diatoms, all $> 5\ \mu m$. Very little difference between taxa was observed with respect to vertical stratification. Small taxonomic changes were observed in the community between May and August, and within each month.

Introduction

With the discovery of the importance in oceanic waters of nanophytoplankton (Lohmann 1920, McAllister et al. 1960) and picophytoplankton (Johnson and Sieburth 1979, Waterbury et al. 1979), it became apparent that the structure of oceanic phytoplankton communities had been incompletely described. The reasons are methodological: when investigators had attempted to include the entire plant community (as opposed to a portion such as diatoms) using visible-light microscopy (e.g. Hasle 1959, Beers et al. 1975, 1982, Fryxell et al. 1979, Booth 1981, Taylor and Waters 1982), the results were biased by inclusion of heterotrophic nanoflagellates $< 5\ \mu m$ (which in most cases could not be differentiated from autophototrophic flagellates) and the omission of cells $< 2\ \mu m$ (picoplankton, which are difficult to distinguish from inorganic particles). Furuya and Marumo (1983) supplemented enumerations of larger cells from light microscopy with estimates of nanophytoplankton derived from the serial-dilution culture-method (most probable number), but they did not include coccoid cyanobacterial picoplankton. Epifluorescence microscopy is a powerful tool for analyzing oceanic phytoplankton communities (Booth 1987), because heterotrophs can be distinguished from photoautotrophs and picophytoplankton can be enumerated (Furuya et al. 1986, Joint and Pomroy 1986).

The subarctic Pacific is a balanced ecosystem, as evidenced by constant phytoplankton stock year-round (McAllister et al. 1960, Clemons and Miller 1984, Sambrotto and Lorenzen 1986, Frost 1987). Nutrients are abundant throughout the year (Anderson et al. 1977), yet phytoplankton stock is low (McAllister et al. 1960). The phytoplankton cells are small (75% of chlorophyll *a* passed a 10 μm filter in June and August of 1959: McAllister et al. 1960), and are hypothesized to be kept in constant check by grazing of zooplankters (McAllister et al. 1960, Heinrich 1962). As part of Project SUPER (Subarctic Pacific Ecosystem Research), the structure of the phytoplankton community has been examined in detail in order to determine the biomass of the various size fractions available as a food source for different sizes of zooplankton. Epifluorescence microscopy, supplemented by scanning electron microscopy (SEM) and inverted light microscopy, was chosen as a tool for investigating the relative biomass of pico-, nano-, and net phytoplankton during spring and summer cruises to two subarctic locations (50°N; 145°W and 53°N; 145°W). The results are reported in the present paper.

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Even though conversions from microscopically determined cell volume to carbon, using factors presently available, are far from perfect (especially for cells smaller than 5 μm), the results are expressed in terms of carbon, because community structure is best understood through biomass not cell concentrations.

Materials and methods

Samples were collected during two cruises to the northeast Pacific Ocean in 1984. Station P, 50°N; 145°W, was occupied from 1–21 May and from 2–12 August, and a more northerly location, 53°N; 145°W, from 13–23 August. Incident light was measured using a LI-COR PAR quantum meter, and water-column attenuation using a LI-COR PAR submersible quantum meter. Temperature and salinity were measured using a CTD, nutrients using an autoanalyzer, chlorophyll *a* by the fluorometric method (Lorenzen 1966). Uptake of ^{14}carbon was estimated at ten depths using an *in-situ* array from midnight to midnight, and integrated for the water column.

Phytoplankton samples for the seven days reported here were collected close to midnight using either 10-liter Niskin bottles on a rosette or 5-liter Niskin bottles attached in series to a hydrographic wire. Water samples (100 ml) were collected from the water bottles as soon as they were on deck and in close sequence with samples for chlorophyll *a* and ^{14}C-uptake studies. At the same time, 250 ml aliquots were collected from the water bottle and fixed with 0.2% (final concentration) formalin. The formalin samples were stored in the dark until examination. The 100 ml samples were stored in well-used polyethylene bottles on ice in the dark and filtered within 4 h: 50 ml was prepared for epifluorescence microscopy (EFM) and 50 ml for scanning electron microscopy (SEM). Samples for SEM were prepared as described in Booth et al. (1982).

Samples for epifluorescence microscopy were fixed and filtered at sea (details in Booth, 1987) and examined ashore. The samples were fixed in dim light with glutaraldehyde (2.5% final concentration) for 20 min in the filter funnel over the filter. The filter apparatus had an effective filter diameter of 13 mm, a stainless steel filter support, a 0.4 μm Nuclepore filter which had been stained with Irgalan black and a Millipore HA backing filter. The sample was filtered at 15 mm Hg until the slide was just dry and then mounted on a glass slide between two drops of immersion oil (Cargille Type B, non-fluorescent). Filters were stored in the dark at 4 °C for a few days, then in a −20 °C freezer until examination. In selected replicate filters examined under green light (Zeiss filter set 48 77 14), counts of *Synechococcus* spp. were the same as counts made under blue light (Booth 1987).

The filters were examined within 3 mo of collection under blue light (Zeiss filter set 48 77 09, reflector 510 nm, excitation 450 to 490 nm, barrier filter at 520 nm) using a 50 W mercury light source. Cells of *Synechococcus* spp. autofluoresced yellow, eukaryotic photoautotrophs red, and some cryptomonads orange. Separate counts were made, in the following order, for: *Synechococcus* spp. (enough fields using 40× objective until at least 300 cells had been counted), eukaryote cells < 2 μm (20 fields using oil immersion 100× objective), 2 to 10 μm photoautotrophs (1 transect using 40× objective) and > 10 μm photoautotrophs (1 to 5 transects using 16× objective). In transects using the 40× objective, from 100 to 500 cells for each size (1 μm increments) were counted. Each cell was measured to the closest 1 μm and assigned to one of the following groups: *Synechococcus,* dinoflagellate, cryptomonad, miscellaneous coccoid and flagellate, or diatom. Dinoflagellates were further divided into spherical, normal (length/width = 2) and slender (length/width = 3) categories in each size range. Cryptomonads were grouped into normal and slender categories. The entire slide was scanned for large diatoms and dinoflagellates using a 16× or 10× objective, or separate counts of cells > 20 μm were made from formalin (0.2%)-preserved samples examined using an inverted light microscope. Diatoms were grouped by eleven shapes.

Enumerations were converted to cells per liter, cell volume per liter and cell carbon per liter, using the following conversions from volume to biomass: *Synechococcus* spp., 0.294 pg C cell^{-1} (Cuhel and Waterbury 1984); cells < 4 μm (other than *Synechococcus* spp.), 0.22 pg C μm^{-3} (Mullin et al. 1966); cells > 4 μm, equations from Strathmann (1967) for converting cell volume (not plasma volume) to cell carbon. Different equations were used for diatoms than for other taxa. Cell volumes in EFM data were not corrected for shrinkage caused by fixation because, although corrections were available for some phytoflagellates 2 to 15 μm (14% of linear dimensions, 33% of volume: Booth 1987), there are no correction factors available for the entire size range of phytoplankton. Therefore, total phytoplankton biomass may be underestimated throughout this study.

Aliquots prepared for scanning electron microscopy (SEM) were critical-point dried from FREON and examined using a JEOL U3 SEM. The relative abundance of five cell types was determined: uniflagellate, biflagellate, green coccoid (wrinkled cell wall in SEM), blue-green coccoid (smooth cell wall in SEM), and miscellaneous coccoids. The assignment of coccoid cells to an algal class was based on comparisons to scanning electron micrographs of cultured species. The size of the living cell was estimated by correcting for the considerable shrinkage resulting from critical-point drying (ca. 42%: see Booth 1987). Estimations of the biomass of each cell type were then made by applying the ratios from SEM enumerations to the total biomass in the 2 to 5 μm size class derived from EFM. For this estimation it was necessary to assume that any heterotrophic cells viewed in SEM had been equally divided between the five cell types.

Since some phytoplankton samples were collected before midnight and some after, I have arbitrarily chosen the date after midnight to label all the figures so that they will correspond with dates of productivity measurements carried out using subsamples from the same bottles.

Table 1. Environmental data from two locations in subarctic Pacific Ocean, in May and August 1984. Data from the mixed layer = *T*: temperature (°C); S: salinity (‰); P: PO_4 (μmol l^{-1}); N: NO_2+NO_3 (μmol l^{-1}); Si: SiO_3 (μmol l^{-1}); Z_m: depth of mixed layer (m); IR: incident radiation (E m^{-2} d^{-1}). Data from the water column = IPP: integrated primary productivity (mg C m^{-2} d^{-1}); 1%: 1% light depth (m); Z_c: depth of chlorophyll maximum (m); Chl_{max}: maximum chlorophyll *a* (μg l^{-1}). 3, 12, 17 May and 4, 9 August: 50°N; 145°W. 15, 20 August: 53°N; 145°W

Date	T[a]	S[a]	P[b]	N[b]	Si[b]	Z_m[a]	IR[c]	IPP[c]	1%[c]	Z_c[c]	Chl_{max}[c]
May 3	6.5	32.6	1.2	12.5	20	36	47.2	270	84	70	0.42
May 12	7.0	32.6	1.0	11.2	19	34	18.5	298	77	40	0.52
May 17	6.9	32.6	1.1	11.0	21	34	27.9	654	70	30	0.96
Aug. 4	12.1	32.4	0.9	7.7	16	10	40.1	353	71	70	0.33
Aug. 9	12.6	32.4	0.9	7.7	17	15	16.2	297	68	80	0.32
Aug. 15	13.4	32.4	1.0	7.9	16	15	26.8	451	69	70	0.29
Aug. 20	13.2	32.5	1.2	11.0	18	20	28.3		65	20	0.38

[a] Data collected by C. Miller
[b] Data collected by P. Wheeler
[c] Data collected by C. Lorenzen and N. Welschmeyer

Results

Environmental data

Selected environmental data collected by other investigators in SUPER is summarized in Table 1. By 3 May the seasonal thermocline was weakly developed around 35 m (Table 1). It deepened to 50 m by 21 May as a result of a storm on 14 May followed by strong winds and high seas from 16 May to 20 May (Denman and Gargett in press). In August, the mixed layer (the isothermal water above the seasonal thermocline, Z_m) was 10 to 20 m deep. Winds were light in August except during a storm on 18 August. The permanent halocline was between 80 and 100 m in both months and nutrients were never limiting. Maximum concentrations of chlorophyll *a* in the water column (chl_{max}) ranged from 0.29 to 0.96 μg l^{-1}.

Phytoplankton biomass

Total phytoplankton biomass is presented for the months of May (Fig. 1) and August (Fig. 2).

Maxima of total phytoplankton biomass (as carbon) were within the seasonal thermocline in May (with the exception of 3 May) and below it in August (Figs. 3a–c and 4a–d). These maxima were well above the 1% light depth in May and at or just above it in August. However, there was considerable algal biomass below the 1% light depth (usually 10 to 26%; 41% on 20 August), and in some cases secondary maxima caused primarily by large diatoms (20 August).

< 2 μm (picoplankton)

Cells under 2 μm averaged 16% of plant carbon in May and 39% in August (Figs. 3a–c and 4a–d). Photoautotrophic eukaryotes (PAE) < 2 μm contributed from 0 to 34% (mean 13% May, 9% August) to picoplankton biomass; the remainder was contributed by species of *Synechococcus* spp. (Figs. 3d–f and 4e–h; note that the abscissas differ from those of Figs. 3a–c and 4a–d). The biomass maxima of picoplanktonic PAE (< 2 μm) *and Synechococcus* spp. usually occurred at the same depth or within 10 m.

In both May and the first half of August, the biomass maximum of *Synechococcus* spp. occurred at the same depth as those of 2 to 5 μm cells and total plant carbon (Figs. 3a–c and 4a–d). In early May and the first half of

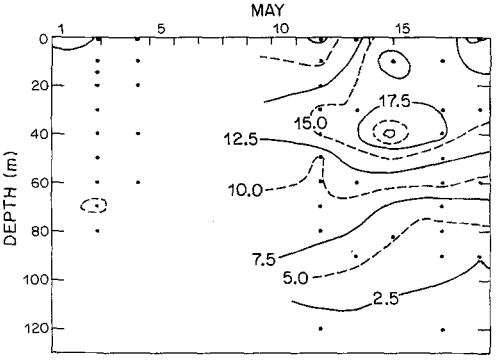

Fig. 1. Phytoplankton carbon (μg C l^{-1}) at Station P in May 1984. Contour intervals = 2.5 μg C l^{-1}

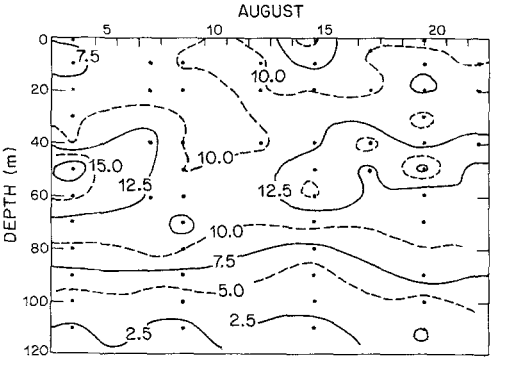

Fig. 2. Phytoplankton carbon (μg C l^{-1}) in August 1984. 3–13 August: Station P, 50°N; 145°W; 14–22 August: 53°N; 145°W. Contour intervals = 2.5 μg C l^{-1}

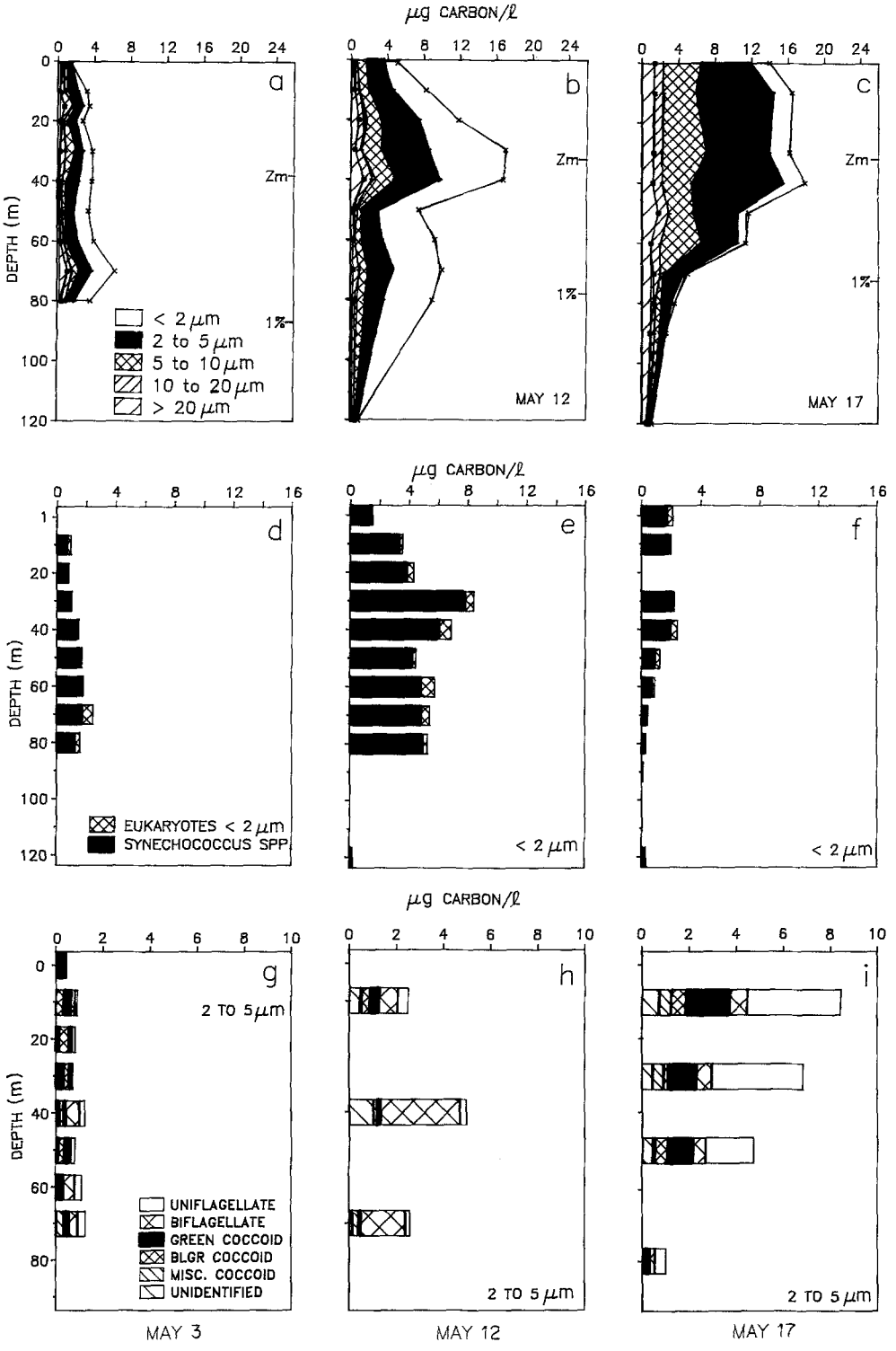

Fig. 3. Distribution of phytoplankton carbon at Station P (50°N; 145°W) in May 1984. (a)–(c) Total phytoplankton carbon divided into five size classes; (d)–(f) cells under 2 μm; (g)–(i) cells 2 to 5 μm. Depth of mixed layer (Z_m) measured at midnight and 1% light depth (1%) the following noon also shown. Note different abscissas between size classes. Size categories in this figure and in Figs. 4–6 are based on maximum dimensions of cells

August, these maxima occurred at, or just above, the 1% light depth whereas later in May they occurred at the bottom of the mixed layer while illumination varied from 2 to 7 E d^{-1} or ca. 10 to 14% of surface illumination. On 20 August, when sampling occurred at 53°N, 145°W, maximum *Synechococcus* spp. biomass occurred at 50 to 60 m, well below the peak of 2 to 5 μm cells at 20 m, the bottom of the mixed layer.

In general, *Synechococcus* spp. were more abundant in August than in May.

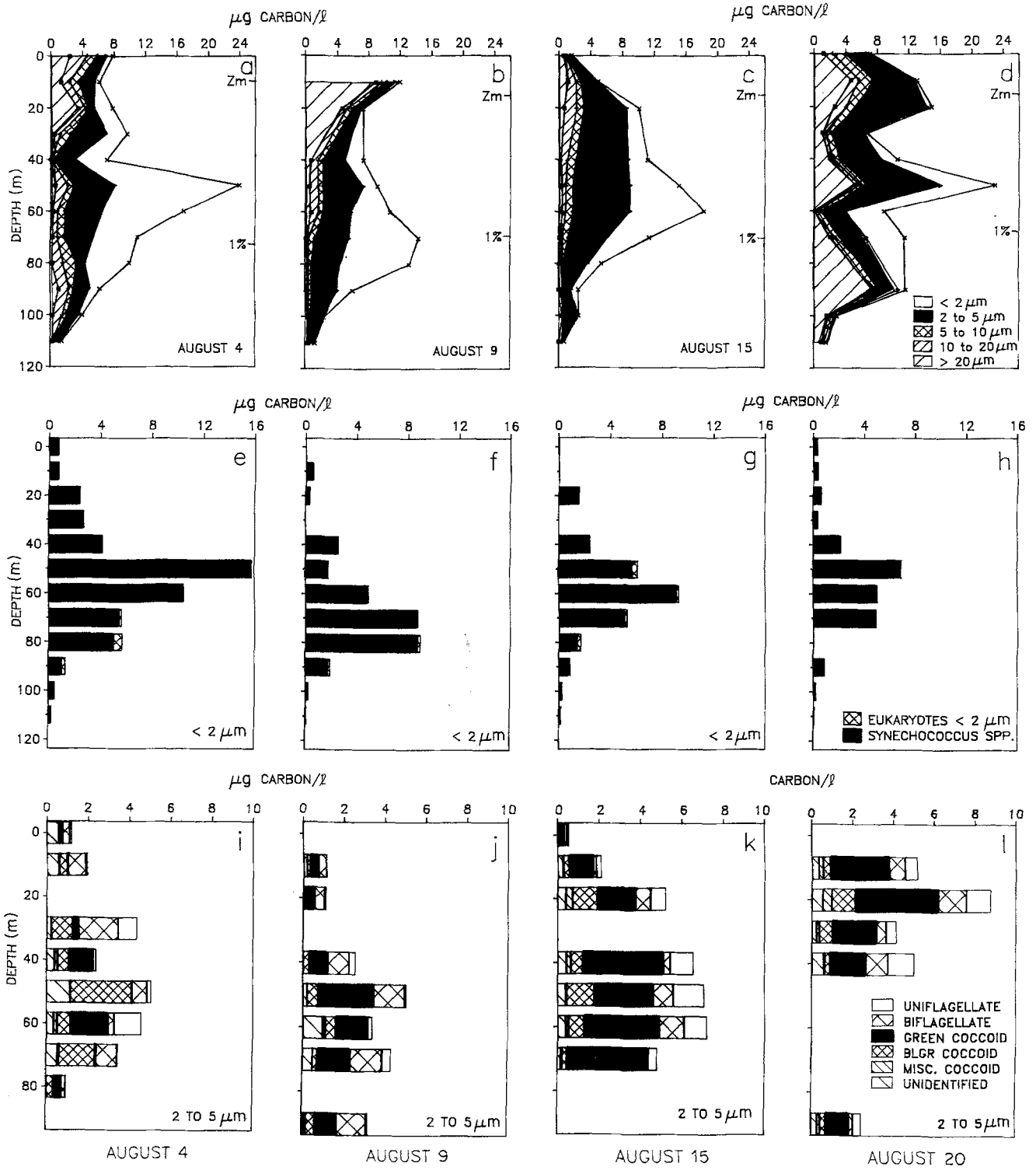

Fig. 4. Distribution of phytoplankton carbon in August 1984. (a)–(d) Total phytoplankton carbon divided into five size classes; (e)–(h) cells under 2 μm; (i)–(l) cells 2 to 5 μm. Z_m and 1% as in Fig. 1. 4 and 9 August: Station P (50°N; 145°W); 15 and 20 August: 53°N; 145°W

2 to 5 μm photoautotrophs

The 2 to 5 μm size class made up about 40% of the plant biomass in May and 37% in August, and biomass maxima coincided with maxima of plant carbon (Figs. 3a–c and 4a–d). Substantial biomass of 2 to 5 μm cells was observed below 80 m whereas biomass of autotrophs < 2 μm (mostly *Synechococcus* spp.) decreased sharply below this depth (Figs. 3c and 4a–d), especially in August. The maximum biomass in the 2 to 5 μm size class occurred in the mixed layer in May and below it in August.

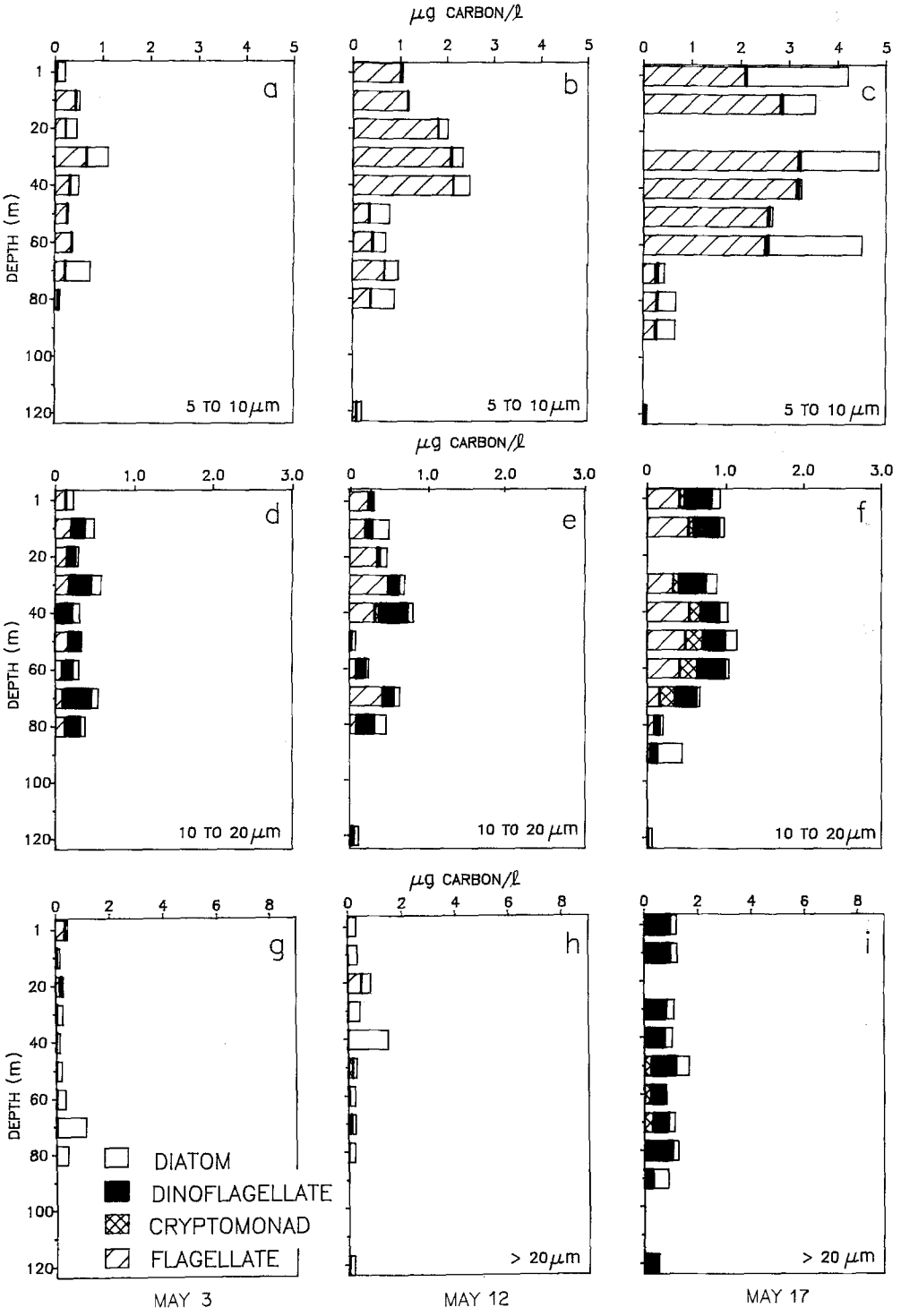

Fig. 5. Distribution of phytoplankton carbon > 5 μm at Station P (50°N; 145°W) in May 1984. (a)–(c) Cells 5 to 10 μm; (d)–(f) cells 10 to 20 μm; (g)–(i) cells > 20 μm

Flagellates dominated this size class in May: biflagellates on 12 May and uniflagellates on 17 May (Fig. 3g–i; note change in abscissas). In August, the predominant forms were coccoid cells (Fig. 4i–l), probably species of Chlorophyceae, based on SEM observations from our culture collection. On 4 August at 50 and 70 m, another type of coccoid cell predominated. These cells were probably cyanobacteria, again based on comparison with SEM observations of blue-greens from culture.

5 to 10 μm photoautotrophs

On 12 May, 5 to 10 μm cells averaged 16% of plant biomass in the upper 40 m, with various types of flagellates dominant (Figs. 5a–c, 6a–d). On 17 May, this size class averaged 25% of plant biomass (Fig. 3c), and the predominant forms were uniflagellates and two small diatoms: *Nitzschia cylindroformis* Hasle and, in lesser numbers, *Cylindrotheca* cf. *fusiformis* Reimann and Lewin. These two

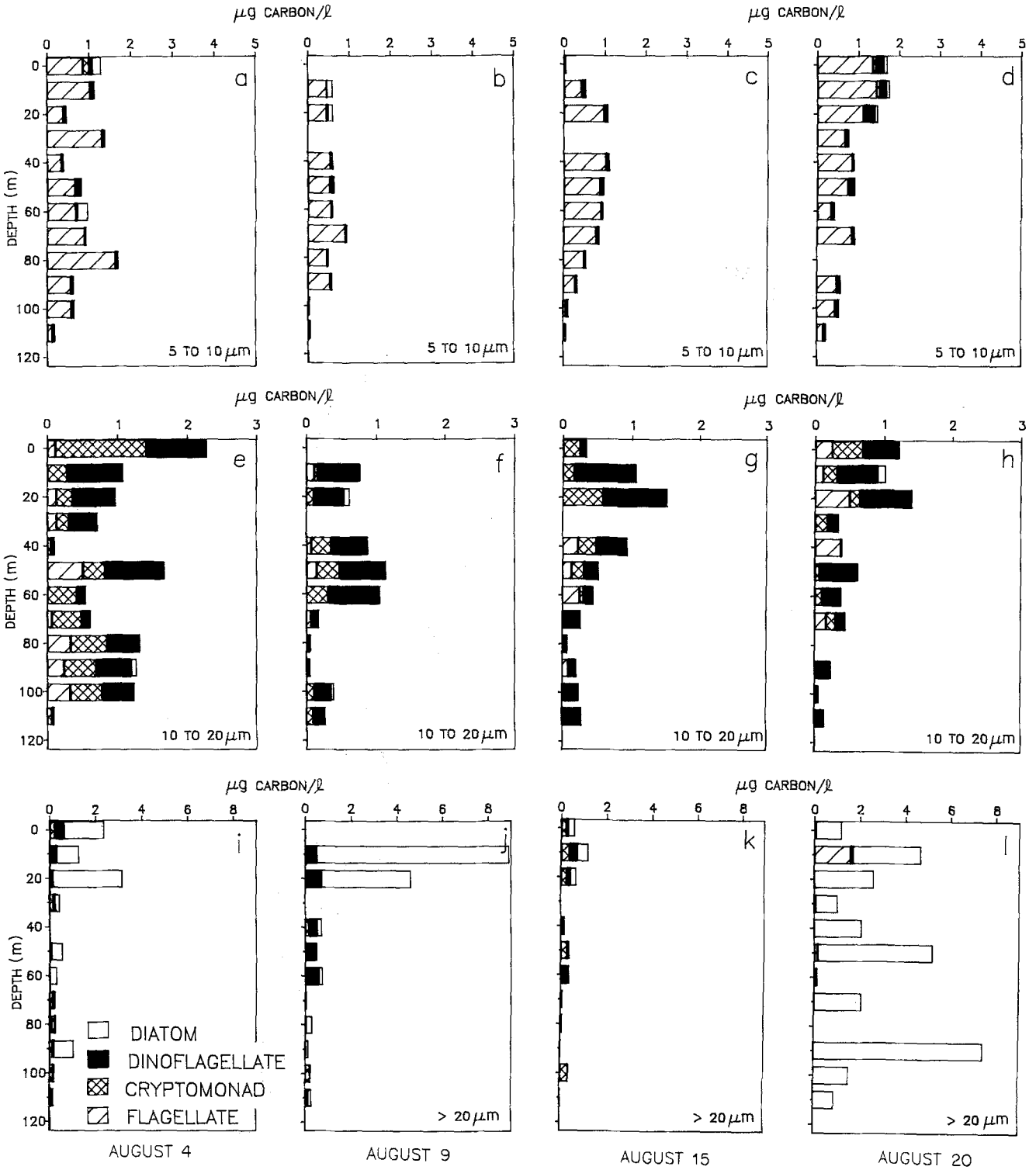

Fig. 6. Distribution of phytoplankton carbon > 5 μm in August 1984. (a)–(d) Cells 5 to 10 μm; (e)–(h) cells 10 to 20 μm; (i)–(l) cells > 20 μm. 4 and 9 August: Station P (50°N; 145°W); 15 and 20 August: 53°N; 145°W

diatoms were present throughout May. Changes in biomass of cells 5 to 10 μm (e.g. 12, 17 May) occurred at the same depths as those of the 2 to 5 μm size class. The 5 to 10 μm size class represented a very small percentage of the total algal biomass in August; flagellates predominated. Cryptomonads and dinoflagellates, 5 to 10 μm, were insignificant in both months.

10 to 20 μm photoautotrophs

The contribution of cells in the 10 to 20 μm size class was small in both months, and biomass in this size class did not vary widely (Figs. 3a–c, 4a–d). In May, dinoflagellates and other flagellates dominated, whereas in August dinoflagellates and cryptomonads were the most important

contributors (Figs. 5 d–f, 6 e–h). A change in cryptomonad biomass was apparent in August.

> 20 μm photoautotrophs

Diatoms dominated the > 20 μm fraction in terms of biomass in both months, except on 17 May when dinoflagellates were predominant (Figs. 5 g–i, 6 i–l). At this time, as well as on 15 August, cryptomonads also made a contribution. Large, unidentified flagellates occasionally contributed to this fraction (3, 12 May and 20 August). Although other diatoms were also present, *Rhizosolenia* spp. were significant in August; they showed high biomass at the surface on 9 August and at 10 and 90 m on 20 August.

Discussion

The present study demonstrates the significance of eukaryotic algae in the lower range of the nanoplankton size class to the phytoplankton community of the open subarctic Pacific. These algae, which measure roughly 2 to 5 μm, occurred throughout the water column in both months sampled in 1984. They reached maximum biomass in the mixed layer in May 1984, but generally peaked below it in August. Together with the picoplankton (the fraction < 2 μm) the total biomass of cells < 5 μm averaged 67% of phytoplankton biomass at Station P in 1984. A similar observation was made by McAllister et al. (1960) in July and August, 1959, at Station P, when 75% of measured chlorophyll *a* passed through a 10 μm mesh filter.

In addition to fitting in with past observations at Station P, the fact that about 56 to 80% of the plant population was < 5 μm in May and August 1984, appears to agree with observations in other temperature and even subtropical oceans (see Table 2). Because live cells are more flexible and can squeeze through smaller filter holes than more rigid preserved cells (Glover et al. 1986 a), cells measuring 2 to 5 μm in the fixed state probably are equivalent in size to living cells which pass alive through a filter with 3 μm holes. Thus, the North Atlantic appears to have the same structure as the subarctic Pacific (Table 2: Glover et al. 1985 b). This generalization is broadened if one can assume that fractionated ^{14}carbon-uptake represents the size structure of the plant community as well as do other methods such as microscopic analysis and chlorophyll *a* fractionations. In the cases where both chlorophyll *a* and ^{14}C-uptake fractionations have been made (Glover et al. 1985 b, Li et al. 1983, Takahashi and Bienfang 1983) this seems to be true; as an example, in the Celtic Sea in December, 1983 (Joint and Pomroy 1986), partitioning of chlorophyll *a* and ^{14}C-uptake was almost identical for the size fractions < 1 μm, 1 to 5 μm, and > 5 μm. Again, the subarctic Pacific and the North Atlantic (see primary production values in Table 2) are similar in the size structure of plant biomass: in the Celtic Sea 20 to 30% ^{14}C was taken up by the < 1 μm fraction, 35 to 40% by cells 1 to 5 μm, and 15 to 20% by cells > 5 μm in summer months (Joint and Pomroy 1983), percentages very similar to those obtained in the present study through microscopic analysis. Tropic waters may have a different structure (Table 2) with a higher percentage of picoplankton (Li et al. 1983), especially in nitrate-depleted waters (Herbland et al. 1985). These are very broad generalizations, expected to have many exceptions. For instance, Joint et al. (1986) found a distinct annual difference in fractionated ^{14}C-uptake in the Celtic Sea. In the present study, as well, although *Synechococcus* spp. contributed on the average 16% to plant carbon in May and 40% in August, the range in the separate samples collected in August was from 4 to 96.7%.

On 16 May 1984, phytoplankton carbon at Station P reached 16.2 μg C l^{-1} at 30 m and the chlorophyll *a* concentration was 0.96 μg l^{-1} (Table 1), more than double the mean value recorded between 1958 and 1974 (Anderson et al. 1977). The highest chlorophyll *a* concentration recorded from surface waters at Station P was 2.08 μg l^{-1}(Anderson et al. 1977). One common assumption is that such brief chlorophyll increases are caused by growth of microplankton (cells > 20 μm), diatoms in particular. Such an assumption is invalidated by the present data in which high chlorophyll *a* concentrations were associated with nanoplankton populations dominated by flagellates and coccoids, not diatoms.

The identity of many of the cells in the 2 to 10 μm size classes remains to be worked out. Reports of oceanic flagellates in this size range are few (Booth et al. 1982, Taylor and Waters 1982, Furuya and Marumo 1983, Murphy and Haugen 1985, Joint and Pomroy 1986), and taxonomic studies fewer (Estup et al. 1984); more taxonomic work has been done on coastal forms (e.g. Moestrup 1979; Hallegraeff 1983). Some species, e.g. cells with loricae or scales, are best identified using TEM, and some, e.g. cryptomonads, using SEM; others must be observed live. In addition to the inherent difficulties of working with live organisms in the nanoplankton size range at sea, many of these species probably will not grow in cultures: Murphy and Haugen (1985) obtained only eight clones of oceanic phytoplankton. For these reasons, oceanic species of nanoplankton, and even larger cells such as naked dinoflagellates and many pennate diatoms, are poorly studied; many are undescribed. A combination, therefore, of EFM, SEM and TEM used on samples prepared at sea may be more successful for analyzing communities of oceanic nanophytoplankton than attempts to obtain cultures.

Many of the cells in the dominant 2 to 5 μm size class of phytoplankton were unidentified coccoid cells, most probably belonging to the Chlorophyceae. In addition to reports of considerable chlorophyll *b* in some oceans (e.g. Jeffrey 1976, Lorenzen 1981, Gieskes and Kraay 1986, Trees et al. 1986, Burkill et al. 1987), a number of qualitative accounts of chlorophycean cocci (similar to *Nanochloris* spp.) from oceanic waters has appeared (Silver and Alldredge 1981, Silver and Bruland 1981, Johnson and Sieburth 1982, Brady 1984, Joint and Pipe 1984; Takahashi

Table 2. Fractionation of phytoplankton in neritic and oceanic waters; *Syn: Synechococcus* spp. Data column shows chl. *a:* chlorophyll *a;* p. prod.: primary production estimated by ^{14}C-uptake; cell nos: cell concentration; biomass: biomass as carbon from cell volume determined microscopically. Su: summer; F: fall; W: winter; Sp: spring

Area, cell size (μm)	%	Data	Season	Comments	Source
Subarctic Pacific					
Station P					
< 10	75	chl. *a*	Su	50°N; 145°W	McAllister et al. (1960) and
< 2	28	biomass	Sp Su		present study
2–5	39				
Transition zone					
< 5	< 35	p. prod.	W Sp Su F	slope waters	Anderson (1965)
North Atlantic					
Celtic Sea					
< 1	20–30	p. prod.	Su ('80)	shelf waters	Joint and Pomroy (1983)
1–5	35–40				
< 1	10–11	p. prod.	Sp ('83–84)	slope waters	Joint and Pomroy (1986)
1–5	7–15				
Gulf of Maine					
< 3	70	chl. *a*	Su	shelf waters	Glover et al. (1985 b)
	64–69	p. prod.			
N.-W. Atlantic					
< 3	84	cell nos	Sp Su F	oceanic	Murphy and Haugen (1985)
< 0.8	75				
Subtropical Pacific					
Kuroshio					
< 4	9	biomass	Su	–	Furuya et al. (1986)
Kuroshio, Oyashio					
Syn.	5–47	chl. *a*	Su	–	Takahashi et al. (1985)
< 3	42–77				
China Sea					
< 3	72–93	chl. *a*	F	neritic	Takahashi and Hori (1984)
Off Hawaii					
< 3	80	chl. *a*	F W	neritic	Takahashi and Bienfang (1983)
< 3	> 80	p. prod.			
< 3	50–85	chl. *a*	W Sp Su F	–	Bienfang et al. (1984)
North Pacific Gyre					
Syn.	45–80	p. prod.	?	35°N; 128°W	Iturriaga and Mitchell (1986)
Subtropical Atlantic					
Gulf Stream					
< 3	82	chl. *a*	Su ('83)	warm-core ring	Glover et al. (1985 b)
< 3	61–79	p. prod.			
1–5	36	chl. *a*	Su ('84)	warm-core ring	Glover et al. (1986 a)
< 5	72	p. prod.			
Tropical Pacific					
Costa Rica Dome					
< 1	20–70	chl. *a*	Sp	upwelling area	Li et al. (1983)
< 1	20–75	p. prod.			
10°N; 93°W					
< 1	50–90	chl. *a*	Sp	non-upwelling	
< 1	18–90	p. prod.			
Tropical Atlantic					
West of Azores					
< 1	40–50	chl. *a*	Su	chl. *a* max. only	Platt et al. (1983)
< 1	60	p. prod.			
Equatorial					
< 1	25	chl. *a*	W Su	nitrate-rich waters	Herbland et al. (1985)
< 1	71			nitrate-deplete waters	
< 1	50			top of nitricline	
Eastern Tropical Atlantic					
< 1	8–67	chl. *a*	F Sp	–	Gieskes and Kraay (1986)
1–3	18–54				
< 1	8–60	p. prod.		–	
1–3	12–18				

and Hori 1984, Murphy and Haugen 1985; Thinh and Griffiths, 1985). Coccoid cells similar to those observed in 1984 (present study) were also present throughout the year in samples collected at Station P from January 1980 through March 1981 and analyzed using only SEM (Booth, unpublished data); they were the predominant organisms (in terms of carbon) in August and September 1980.

On the days sampled during the present study, cells of *Synechococcus* spp. occurred throughout the water column, with maxima in the mixed layer well above the 1% light depth in May (except on 3 May when the maximum was at 70 m close to the 1% light depth) and below the mixed depth close to the 1% light depth in August. A major conclusion of the present study is that *Synechococcus* spp. can be abundant in the upper euphotic zone (a conclusion also reached by Joint and Pomroy 1986) as well as in deeper waters near the bottom of the euphotic zone (Glover et al. 1985a). Especially in August (Fig. 4a–d), it is also clear that some autotrophic eukaryotes of 2 to 5 μm are abundant even deeper than *Synechococcus* spp., as described by Glover et al. (1986b).

Photoautotrophic eukaryotes $< 2\ \mu$m never contributed more than 34% (mean = 12% in May, 9% in August) to picoplankton biomass, and therefore made negligible contributions to total plant carbon at Station P in May and August 1984. Previous predictions of the importance of this group of plants were based on cell concentrations of 5×10^5 cells l^{-1} of *Micromonas pusilla* (Butch.) Manton and Parke in the North Sea (Knight-Jones and Walne 1951) and 10^6 to 10^7 cells l^{-1} of a non-motile prasinophyte over the Grand Banks (Johnson and Sieburth 1982). Although similar concentrations were observed at Station P (maxima for eukaryotes $< 2\ \mu$m in both May and August were 5×10^6 cells l^{-1}), conversion of cell counts to biomass diminished their apparent importance. *M. pusilla* is hardy in culture (Knight-Jones and Walne, 1951); it is well preserved in glutaraldehyde (Booth 1987). Yet in samples prepared as whole mounts at Station P in 1980, 1983 and 1984, and viewed using transmission electron microscopy (TEM), only eight specimens of *M. pusilla* were seen. Similarly, no scaled spheroids, 0.5 to 1.0 μm, similar to those described by Johnson and Sieburth (1982) were seen. It may be that both these forms are more common in coastal than in oceanic waters, as observed by Knight-Jones and Walne (1951) and Johnson and Sieburth (1982). In summary, the photoautotrophic eukaryotes smaller than 2 μm in the subarctic Pacific could not be identified; they were not very important in terms of carbon in 1984 and, when abundant, occurred close to the 1% light depth.

There is no evidence in the present data for a two-layered system (i.e., differences between depths in species assemblages) within the phytoplankton community. It is possible that phytoplankton *species* occurred in distinct layers but that these layers were not apparent in the analysis of *genera* and size classes. Such layers, however, would be difficult to observe given the difficulty of identifying species of oceanic nanoplankton. Layered systems have been described with species that can be identified using light microscopy (e.g. Venrick 1982, Furuya and Marumo 1983). Furuya and Marumo also analyzed the nanoplankton community (fragile cells only) using serial-dilution cultures. Although they concluded that the nanoplankton genera in the deep layer were different from those in surface waters, this conclusion seems to be based on insufficient evidence given the low cell counts. In conclusion, differences between depths in the species assemblages of pico- and nanoplankton forms (60% of oceanic phytoplankton biomass in this study) have not been demonstrated, and such demonstration will be very difficult to achieve.

In general in 1984 at Station P, flagellates dominated the 5 to 10 μm size class, flagellates and dinoflagellates the 10 to 20 μm size class, and diatoms the $> 20\ \mu$m size class. In May, diatoms 5 to 10 μm were co-dominant with the flagellates, whereas in August they were not. The diatoms in this group were dominated by one species, *Nitzschia cylindroformis* Hasle, a form observed so far only from the subarctic Pacific and often present in large numbers (Hasle and Booth 1984).

In the 10 to 20 μm size class, cryptomonads were co-dominant with dinoflagellates in August, but were less important in May. Although cryptomonads can successfully utilize very low light levels (Stewart and Wetzel 1986), in August 1984 their maximum biomass was at the surface at Station P. Their biomass may have been underestimated in this study due to loss of fluorescence during collection (Exton et al. 1983) or upon fixation (see Booth 1987). Because of their general fragility upon fixation with formalin (Booth 1987), the occurrence of cryptomonads in oceanic waters has probably been underestimated. A limited number of cryptomonad species (those that are preserved in formalin) have been reported by Hasle (1959) from Antarctic waters and by Beers et al. (1975, 1982) from the Central Pacific. More species are reported from samples observed live (Throndsen 1976) or fixed in Lugol's (Taylor and Waters 1982) or glutaraldehyde (Booth et al. 1982).

Small flagellates (including "monads") and non-thecate dinoflagellates comprised the major portion (45% and 24 to 32%, respectively) of plant carbon in the North Pacific central gyre in June, 1973 (Beers et al. 1975) and larger portions in other seasons (Beers et al. 1982). It is difficult to compare these data with mine because both phototrophic and heterotrophic flagellates were included in the data from the central gyre and because cells of *Synechococcus* spp. were not enumerated, but it would appear that the phytoplankton community in the North Pacific central gyre may include more non-thecate dinoflagellates (see also Fryxell et al. 1979) than that of the subarctic Pacific.

Because the present study is based on a new way of determining phytoplankton biomass, it is important to emphasize the essential parts of the technique. The preparation of slides for epifluorescence was performed at sea, and fixed, not live, cells were filtered to minimize cell loss on contact with the filter. The cells were fixed over the filter just before filtration to minimize loss to container walls and aggregation of cells resulting from mucus secretion (Booth et al. 1982). Stainless steel filter supports were faster

than sintered glass supports. A mercury light source produced the intense beam necessary to maximize autofluorescence after storage of the slides (Booth 1987).

The accuracy of the biomass estimates based on EFM enumerations has been discussed elsewhere (Booth, 1987). One additional source of error could be the factors used for conversion of cell volume to cell carbon. Experimental determinations of the carbon content of *Synechococcus* spp. cells (0.40 pg C μm^{-3}: Takahashi et al. 1985; 0.294 pg C $cell^{-1} = 0.56$ pg C μm^{-3} for cell 1 μm in diameter: Cuhel and Waterbury 1984) show larger values than would be expected from theoretical considerations (0.121 pg C μm^{-3}: Watson et al. 1977, Ituriaga and Mitchell 1986). I used the factor of Cuhel and Waterbury because of its demonstrated reliability; very likely it overestimates *Synechococcus* spp. biomass. For the porposes of this paper such an overestimate only more strongly emphasizes the greater ecological importance of small photoautotrophic eukaryotes, 2 to 5 μm, relative to prokaryotic picoplankton.

Conversions from volume to biomass for small cells are presently based on very few data: the smallest cell used in developing the Strathmann equations (1967) was 3.0 μm in diameter (*Emiliania huxleyi*). When these equations are extrapolated to cells < 3 μm, the carbon:volume ratios increase rapidly (e.g. 4 μm, C:vol = 22%; 3 μm, C:vol = 24%; 2 μm, C:vol = 29%; 1 μm, C:vol = 38%). Therefore, for cells < 4 μm (other than *Synechococcus* spp. cells) I used a constant conversion factor of C:vol = 0.22 which is the Strathmann ratio for a 4 μm cell (suggested by F. Reid, personal communication, also see Mullin et al. 1966 and Borsheim and Bratbak 1987). There is now a real need for more extensive determinations of the carbon content of phytoplankton cells, both eukaryotes and prokaryotes, under 4 μm in diameter.

Acknowledgements. This work was supported by National Science Foundation Grants OCE 83-10827 and OCE 82-14164. R. Horner enumerated the phytoplankton > 20 μm in the May samples. SEM enumerations were performed at the Botany/Quaternary Research Center Electron Microscope Laboratory at the University of Washington. I thank K. Banse, B. Frost, R. Horner and J. Lewin for helpful discussions; M. Strom, R. Hu, J. Mueller and S. Stanton for technical assistance; the captain and crew of the R.V. "Wecoma" for assistance at sea; and all the other investigators in project SUPER for their cooperation.

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Date of final manuscript acceptance: October 8, 1987.
Communicated by P. C. Schroeder, Pullman