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CHARACTERIZATION OF OCEANIC PHOTOSYNTHETIC PICOEUKARYOTES BY FLOW CYTOMETRY[1]

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ABSTRACT

To interpret flow cytometric data that are routinely obtained on natural oceanic communities, 23 strains of photosynthetic picoeukaryotes belonging to four classes (Prasinophyceae, Chlorophyceae, Pelagophyceae, and Prymnesiophyceae) and six pigment types were investigated for their light scattering in the forward and right-angle directions, chlorophyll fluorescence, and DNA content as measured by flow cytometry. Cell size was assessed by Coulter counter, and pigment composition was measured by reverse-phase high-performance liquid chromatography. The size and GC% of the nuclear genome of cultured picoeukaryotes was measured from the fluorescence of DNA-specific dyes. Using these two parameters, we could discriminate species within pigment groups. DNA staining of preserved natural samples may also prove useful in discriminating cooccurring populations in situ *as long as the communities are not too complex. Using the relationships that we established between size and light-scattering properties of the cells, we estimated equivalent diameters of picoeukaryotes in natural populations to be between 1.3 and 2 μm. Chlorophyll* a *content was between 6 and 16 fg·cell^{-1} as calculated from relationships that we established between chlorophyll* a *content and red fluorescence of the cultured strains. With respect to size, chlorophyll* a *content, and pigment composition,* Pelagomonas *sp. strains (Pelagophyceae) appeared to be the most representative of the natural communities in subtropical ocean waters. In contrast, green coccoid strains, which often outcompete other strains in culture, might only be minor contributors to these communities.*

Key index words: DNA content; flow cytometry; picoplankton; pigments

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The term picoplankton was originally proposed to identify the bacterioplankton of the size class less than 2 μm (Sieburth et al. 1978). It was later extended to all photosynthetic organisms in this size class (Johnson and Sieburth 1982). A definition based on operational criteria more useful in field studies (i.e. the ability of cells to pass through 3-μm-pore-size filters) has been adopted by numerous authors (Takahashi and Bienfang 1983, Takahashi and Hori 1984, Murphy and Haugen 1985, Li 1986). Photosynthetic picoplankton include *Synechococcus* cyanobacteria (Waterbury et al. 1979), eukaryotes (Johnson and Sieburth, 1982), and *Prochlorococcus* prochlorophytes (Chisholm et al. 1988). These organisms may contribute significantly to standing stock and primary production in the oligotrophic areas of the world oceans (Li and Platt 1987, Raimbault et al. 1988). Their vertical distribution in the euphotic zone has been studied by epifluorescence microscopy (Murphy and Haugen 1985, Le Bouteiller et al. 1992) or flow cytometry (Olson et al. 1985, Li and Wood 1988). Field data indicate a typical abundance in cell·mL^{-1} of 10^5 prochlorophytes, 10^{3-4} *Synechococcus*, and 10^3 picoeukaryotes in open ocean waters (Murphy and Haugen 1985, Li and Platt 1987, Li and Wood 1988, Campbell and Vaulot 1993, Campbell et al. 1994a). Although picoplankton can also be important at times in coastal waters (Courties et al. 1994), we will focus, in what follows, on oligotrophic ocean waters.

While both *Synechococcus* and *Prochlorococcus* have received a lot of attention, picoeukaryotes have been the object of fewer studies. Some basic questions such as "What are the major classes that dominate eukaryotic picoplankton?" or "Are the species isolated in culture representative of the oceanic environment?" are still largely unanswered (Campbell et al. 1994b). The recent description of a new class of algae, the Pelagophyceae (Andersen et al. 1993), based on a novel picoplankton species typifies the huge amount of work still to be done to assess the diversity of this community.

Reverse-phase high-performance liquid chromatography (HPLC) analysis of taxon-specific pigments and their ratios to total chlorophyll (Chl) *a* suggest that prymnesiophytes, chrysophytes, and, at certain times, prasinophytes and chlorophytes are the major contributors to the eukaryotic phytoplankton biomass in the euphotic zone of oligotrophic regions of the Atlantic and Pacific Oceans (Hooks et al. 1988, Everitt et al. 1990, Williams and Claustre 1991, Barlow et al. 1993, Letelier et al. 1993). However, there are limitations in the use of specific marker pigments : Chl *a* ratios for quantitative and qualitative studies because most of these markers are not restricted to a single group and often have a scattered distribution among species of that group. Moreover, because accessory pigments : Chl *a* ratios are derived from culture data, they can only be considered as approximations in the absence of values from the field samples.

Scanning and transmission electron microscopy (SEM and TEM) are the only methods by which morphological and ultrastructural details can be studied and reliable cell-by-cell identification made, but they provide only qualitative information. Some species and morphological types, such as *Micromonas pusilla* (Butcher) Manton et Parke, a scale-bearing prasinophyte (probably *Bathycoccus prasinos* Eikrem et Throndsen), *Chlorella*-like, and *Nannochloris*-like cells, as well as unidentified small chryosophytes and prymnesiophytes, have been widely observed with these techniques in oceanic waters of the North Atlantic (Johnson and Sieburth 1982, Estep et al. 1984), Celtic Sea (Joint and Pipe 1984) and North and Central Pacific (Takahashi and Hori 1984, Hoepffner and Haas 1990). A number of new species was also described solely from TEM and SEM observations of field samples, while attempts to culture these organisms failed. For instance, seven species of Parmales (Chrysophyceae) were described from subarctic Pacific and Antarctic waters by Booth and Marchant (1987), and *Aureococcus anophagefferens* Hargraves et Sieburth was described by Sieburth et al. (1988) from Narragansett Bay sea water. Nevertheless, small eukaryotes are often referred to as unidentified coccoids and flagellates (Booth and Marchant 1987, Hoepffner and Haas 1990) because they preserve poorly and lack taxonomically useful morphological features. Some delicate groups, like the chrysophytes, cryptophytes, and prasinophytes, are probably underrepresented in preserved samples (Murphy and Haugen 1985, Shapiro and Guillard 1986). Furthermore, it is likely that some important components of this size class have not yet been characterized, as many of them are not easy to grow in culture. Thus, both HPLC and microscopy present limitations to the study of picoeukaryotic populations whose taxonomy is still poorly understood.

To circumvent inherent problems of these traditional techniques, attempts have been made to develop new approaches, like discrimination of cells from apparent size and shape of their chloroplast (Li and Wood 1988) or immunofluorescence recognition of species (Shapiro et al. 1989a, b) by epifluorescence microscopy. While the former technique has very poor taxonomic resolution, immunofluorescence was successfully used by Campbell et al. (1994b) for the study of natural communities. However, only a small percentage of the total population was labeled by available antibodies, suggesting that the strains probed (e.g. *Pycnococcus provasolii* Guillard strain Ω48–23 [= CCMP 1203] and *Pelagococcus subviridis* Norris [clone PELA CL2]) are not really representative of oceanic communities.

In the past decade, flow cytometry has gained wide acceptance as a method of estimating abundances of the different populations of picoplankton and obtaining general information on cell size and pigment content from light scattering and red (Chl) or orange (phycoerythrin) fluorescences (Li and Wood

1988, Olson et al. 1990b, Li et al. 1992, Campbell and Vaulot 1993, Veldhuis et al. 1993). Good interpretation of flow cytometric data for *Prochlorococcus* and *Synechococcus* has been achieved (Olson et al. 1990a, b, Veldhuis and Kraay 1990). In contrast, picoeukaryotes have received much less attention. The interpretation of flow cytometry data for these organisms may provide information on some of the outstanding questions alluded to earlier. To answer these questions, we examined flow cytometry signatures (scatter, Chl fluorescence, and DNA fluorescence) of 23 cultured picoeukaryote strains belonging to a wide taxonomic range as shown by HPLC signatures, TEM observations, and genome size measurements. These flow cytometric signatures were calibrated to cell size, Chl *a* content, and DNA content. Calibrations were then used to interpret flow cytometry data on field populations from various oceanic regions.

MATERIALS AND METHODS

Data concerning origin and isolation conditions of strains are provided in Table 1. For isolation of novel strains (ALMO, EUM, MAX, currently maintained in Roscoff), sea water was sampled during three different French cruises and filtered through 0.6- or 1-μm Nuclepore filters either on the ship or back in the laboratory. Different culture conditions were tested in an attempt to select different algal groups. Stock solutions, corresponding to K/10 medium (Keller et al. 1987) or a modification of this medium with 50 μM NH_4Cl and 50 μM urea as the N source and trace metals, used usually for *Prochlorococcus* cultures (Chisholm et al. 1992), were added to filtered sea water. Cultures were maintained in low blue light (10 μmol quanta·m^{-2}·s^{-1}, EUM 8, 10, 13B, 16B, 19, MAX 71, 72, 73) or high white light (50 μmol quanta·m^{-2}·s^{-1}, ALMO 1, 2, EUM 18, 13A, 16A). White light was provided by Sylvania Daylight fluorescent bulbs, and blue light was obtained by filtration through blue Plexiglas (Rohm and Haas, Blue 2424). The eukaryotic strains were separated from prochlorophytes, cyanobacteria, and larger phytoplanktonic cells using either serial dilution, antibiotic treatment (ampicillin, 1 μg·mL^{-1} of medium), or cell sorting by flow cytometry. Once established, the cultures were transferred to K medium at 17° C and at the light irradiance used for isolation.

A number of picoeukaryote strains were purchased from the Provasoli-Guillard Culture Centre for Marine Phytoplankton (CCMP, Bigelow Laboratory for Ocean Sciences, Maine). *Imantonia* sp. was provided by the Plymouth Culture Collection (PCC, Plymouth Marine Laboratory, Plymouth, U.K.), and *Bathycoccus prasinos* (type strain) was kindly provided by Dr. W. Eikrem (University of Oslo, Blindern). They were cultured in K medium at 17° C and 50 μmol quanta·m^{-2}·s^{-1}, white light.

Pigment analysis. For each strain, 20–30 mL of exponentially growing culture was filtered through 25-mm Whatman GF/F filters, immediately frozen in liquid nitrogen, and transferred at −80° C until analysis by reverse-phase HPLC as described by Barlow et al. (1993). Cells were counted using an Epics 541 flow cytometer equipped with a microsample delivery system (Coulter, Hialeah, Florida). Pigments were identified by comparison with retention times of various pigments present in well-documented species and diode array spectrophotometry (Waters 990 detector).

Staining of nuclei. Cells were lysed by the addition of a buffer (9:1, v:v) containing 30 mM $MgCl_2$, 20 mM NaCitrate, 0.12 M sorbitol, 55 mM HEPES, 5 mM EDTA, 5 μL·mL^{-1} β-mercaptoethanol, and 0.1% triton X-100. In these conditions, nuclei were stable for at least 30 min. An aliquot of the nuclei suspension was stained with 5 μg·mL^{-1} Hoechst 33342 (HO; Sigma B-2261) that binds to AT-rich DNA regions. A second aliquot was treated with 0.01% RNAse (i.e. a mixture of RNAse A and RNAse B, Sigma R-4875 and R-5750, 1:1, w:w) and stained with 30 μg·mL^{-1} propidium iodide (PI; Sigma P-5264), an intercalating dye that binds to all nucleic acids. Freshly extracted nuclei of *Phaeocystis* sp. (strain PCC 64, PCC) were used as an internal standard (210 fg of nucleic DNA, GC% = 54, Vaulot et al., unpubl.). Samples were analyzed by flow cytometry using either the ultraviolet (353–357 nm) (HO) or the 488-nm (PI) lines of the laser (Coherent, Palo Alto, California). Emitted fluorescence was collected through a 485–nm band-pass filter or a 610-nm long-pass filter for HO and PI, respectively, and recorded for processing using CYTO PC (Vaulot 1989). The average HO-DNA and PI-DNA fluorescence of the picoeukaryote populations were computed and normalized to the fluorescence of *Phaeocystis* nuclei. The level of nuclear DNA (in femtograms per nucleus) was calculated as follows: $[DNA] = R_{PI} \times 210$. GC% was estimated using the R_{PI}:R_{HO} ratio, following the model developed by Godelle et al. (1993). R_{PI} and R_{HO} are the normalized values of DNA-HO and DNA-PI fluorescence, respectively.

Staining of whole cells. For a number of strains and for the analysis of natural sea water samples, the preceding protocol was inefficient, either because cells were not lysed in the buffer (CCMP 253, 515, 1127, 1192, 1199, 1203) or because nuclei could not be discriminated from bacteria and background noise (EUM 10, 13, 16, 18, 19, natural samples). We therefore performed DNA staining on 0.5% paraformaldehyde–preserved cells that were diluted in 0.22-μm-filtered sea water (1:9, vol:vol). Fixation preserved Chl fluorescence and thus allowed the discrimination of photosynthetic cells from bacteria. We used either HO or the GC-specific dye Chromomycin A3 (CA3, Sigma C 2659), because PI did not penetrate into fixed cells. HO (1 μg·mL^{-1}) or CA3 (30 μg·mL^{-1}, together with 30 mM $MgCl_2$ and 0.01% RNAse) was added to the diluted samples that were incubated for 1 h at 37° C and analyzed by flow cytometry. Preserved cells of *Phaeocystis* sp. (PCC64) were used as an internal standard. Rp_{HO}, Rp_{CA3}, the normalized HO, and CA3 fluorescence of the preserved picoeukaryotes were calculated. GC% of total DNA was estimated from Rp_{HO}/Rp_{CA3}, following the model developed by Godelle et al. (1993).

Volumes, forward and right-angle light scatter, and red fluorescence. Volume in either exponential or early stationary phase was measured with a Coulter counter (Multisizer II) equipped with a 15-μm-orifice tube and calibrated with 1.98-μm beads (Polysciences, Warrington, Pennsylvania, batch No. 405971). Live cells were diluted in filtered sea water to a final concentration of 10^4 cells·mL^{-1}. For each strain, three samples of 5000–10,000 cells were analyzed.

Forward and right-angle light scatter (FALS and RALS) and chlorophyll fluorescence were measured with an Epics 541 flow cytometer (Coulter, Hialeah, Florida) equipped with a Biosense flow cell and confocal lens to increase sensitivity (Olson et al. 1990a, Vaulot et al. 1990). Samples were illuminated with an argon laser set at 488 nm. Analysis was performed on live and preserved cells. The latter were fixed for 15 min in 0.5% paraformaldehyde (Sigma P-6148, St. Louis, Missouri) at room temperature and deep frozen in liquid nitrogen. They were maintained at −80° C until thawed for analysis. All measurements were normalized to 0.95-μm yellow-green fluorescent beads (Polysciences, lot No. 71825).

Field samples. Samples were collected during several cruises (see later and Table 6). Most of them were fixed on board with 1% glutaraldehyde, deep frozen in liquid nitrogen, and stored at −80° C until analysis by flow cytometry. Samples collected in January 1989 from the northwest Mediterranean Sea were analyzed on board immediately. RALS and red fluorescence signals of picoeukaryote populations were measured with the same protocol as for cultured cells. These parameters were normalized to 0.95-μm beads (Polysciences) to allow comparison with cultured strains, and average values of RALS and red fluorescence were calculated for samples of each oceanic region.

TABLE 1. *Classification, strain code, and origin of the picoeukaryotic species surveyed. na = information not available.*

					Origin				Isolation conditions	
	Species	Strain	Flagella	Area	Latitude	Longitude	Date	Depth (m)	Medium	Irradiance (μmol quanta $m^{-2} s^{-1}$)
Chlorophyceae	*Chlorella* sp	CCMP 253	None	Narragansett, RI	na	na	Jun 1986	na	na	na
	Nannochloris sp.	CCMP 515	None	Cape Bolinas, Philippines	na	na	Feb 1987	na	na	na
	Unidentified	CCMP 1127	None	Gulf of Mexico	na	na	Feb 1981	na	na	na
Prasinophyceae	*Micromonas pusilla*	CCMP 490	1	Woods Hole, MA	na	na	Jun 1964	na	na	na
	Bathycoccus prasinos	Type strain	None	Gulf of Naples	40°48′ N	14°16′ E	Apr 1986	100	Erd-Schreiber	106
	Bathycoccus prasinos	ALMO 1	None	Alboran Sea	36°11′ N	01°51′ W	May 1991	Surface	K/10	50
	Bathycoccus prasinos	ALMO 2	None	Alboran Sea	36°11′ N	01°51′ W	May 1991	Surface	K/10	50
	Unidentified	EUM 10	None	Tropical Atlantic	21°02′ N	31°08′ W	Oct 1991	110	Modified K/10	50
	Unidentified	EUM 13A	None	Tropical Atlantic	21°02′ N	31°08′ W	Oct 1991	120	Modified K/10	50
	Unidentified	EUM 13B	None	Tropical Atlantic	21°02′ N	31°08′ W	Oct 1991	120	Modified K/10	10
	Unidentified	EUM 16A	None	Tropical Atlantic	21°02′ N	31°08′ W	Oct 1991	105	Modified K/10	50
	Unidentified	EUM 16B	None	Tropical Atlantic	21°02′ N	31°08′ W	Oct 1991	105	Modified K/10	10
	Unidentified	EUM 18	None	Tropical Atlantic	20°25′ N	31°08′ W	Oct 1991	80	Modified K/10	50
	Unidentified	EUM 19	None	Tropical Atlantic	20°25′ N	31°08′ W	Oct 1991	120	Modified K/10	10
	Pycnococcus provasolii	CCMP 1203	None	Old WHOI sta II	38°20′ N	69°35′ W	Jul 1978	30	na	na
	Pycnococcus sp.	CCMP 1192	None	Oceanus 83 sta II	38°42′ N	72°22′ W	Jul 1980	20–25	na	na
	Unidentified	CCMP 1199	None	Oregon 2 sta 6	25°25′ N	87°00′ W	Apr 1980	Surface	na	na
Pelagophyceae	*Pelagomonas* sp.	EUM 8	1	Tropical Atlantic	19°42′ N	25°52′ W	Oct 1991	100	K/10	10
	Pelagomonas sp.	MAX 71	1	Sargasso Sea	26°18′ N	63°26′ W	Oct 1987	140	K	4
	Pelagomonas sp.	MAX 72	1	Sargasso Sea	26°18′ N	63°26′ W	Oct 1987	140	K	4
	Pelagomonas sp.	MAX 73	1	Sargasso Sea	26°18′ N	63°26′ W	Oct 1987	140	K	4
Prymmnesiophyceae	Unidentified	CCMP 625	None	na	na	na	na	na	na	na
	Imantonia sp.	PCC 18561	2	Northeast Atlantic	20° W	52° N	Jun 1989	30	K	40

TABLE 2. *Pigments of picoeukaryotic strains; ratios of main accessory pigments to Chl* a *and Chl* a *content per cell for selected strains. Mg-2,4-D = Mg-2,4-divinyl phaeoporphyrin* a_5 *monomethyl ester; nd = not detected; na = not available; — = peak unresolved.*

	Species	Strains	Chl *a* (fg·cell^{-1})	Accessory pigments to Chl *a* ratios						Pigment group
				Chl *b*	Zeaxanthin	Lutein	Prasinoxanthin	Violaxanthin	Mg-2,4-D	
Chlorophyceae	*Chlorella* sp.	CCMP 253	27.6	0.381	nd	0.231	nd	nd	nd	I
	Nannochloris sp.	CCMP 515	95.0	0.241	nd	0.247	nd	nd	nd	I
	Unidentified	CCMP 1127	26.2	0.461	nd	0.207	nd	nd	nd	I
Prasinophyceae	*Micromonas pusilla*	CCMP 490	13.0	0.843	0.023	nd	0.059	0.133	0.032	IIA
	Bathycoccus prasinos	Type strain	na	0.532	0.032	nd	0.186	0.086	0.032	IIA
	Bathycoccus prasinos	ALMO 1	4.5	0.482	0.028	nd	0.165	0.060	0.027	IIA
	Bathycoccus prasinos	ALMO 2	na	0.522	0.016	nd	0.154	0.081	0.032	IIA
	Unidentified	EUM 10	na	0.719	0.008	nd	0.175	0.054	nd	IIA
	Unidentified	EUM 13A	3.8	0.785	0.021	nd	0.131	0.054	0.012	IIA
	Unidentified	EUM 13B	4.6	0.958	0.002	nd	0.142	0.069	0.010	IIA
	Unidentified	EUM 16A	5.2	1.044	0.017	nd	0.226	0.042	0.020	IIA
	Unidentified	EUM 16B	4.0	1.009	0.002	nd	0.151	0.054	0.011	IIA
	Unidentified	EUM 18	2.5	0.915	0.052	nd	0.287	0.134	0.031	IIA
	Unidentified	EUM 19	7.9	1.029	—	nd	0.214	0.032	0.017	IIA
	Pycnococcus provasolii	CCMP 1203	105.9	0.956	0.094	nd	0.526	—	0.111	IIB
	Pycnococcus sp.	CCMP 1192	na	0.952	0.005	nd	0.377	—	0.255	IIB
	Unidentified	CCMP 1199	161.6	0.913	0.080	nd	0.474	—	0.052	IIB
				Chl $c_{1,2}$	Chl c_3	Fucoxanthin	19′-HF	19′-BF	Diadinoxanthin	
Pelagophyceae	*Pelagomonas* sp.	EUM 8	17.4	0.129	0.059	0.097	nd	0.677	0.001	IV
	Pelagomonas sp.	MAX 71	na	0.156	0.079	0.069	nd	0.947	0.030	IV
	Pelagomonas sp.	MAX 72	13.1	0.245	0.119	0.080	nd	1.532	0.020	IV
	Pelagomonas sp.	MAX 73	na	0.206	0.082	0.122	nd	1.075	0.040	IV
Prymnesiophyceae	Unidentified	CCMP 625	119.0	0.192	0.106	0.062	2.514	nd	0.002	III
	Imantonia sp.	PCC 18561	na	0.044	0.090	0.078	0.517	0.178	0.058	V

The staining protocol designed for preserved cultured picoeukaryotes was tested for natural populations. Surface sea water was collected off Roscoff, in the western English Channel, fixed with paraformaldehyde, and stained with HO and CA3 as already described. For each group that was distinguished on biparametric cytograms showing DNA-fluorochrome fluorescence vs. Chl fluorescence, Rp_{HO} and Rp_{CA3} were calculated.

RESULTS AND DISCUSSION

Selection of strains. We tried to obtain a collection of picoeukaryote strains as complete as possible and as representative as possible of natural communities. First, we included species that have often been observed in oligotrophic areas or have frequently been isolated from the ocean such as *Micromonas pusilla* (Throndsen 1976, Johnson and Sieburth 1982, Furuya and Marumo 1983, Hoepffner and Hass 1990), *Pycnococcus provasolii* (Guillard et al. 1991), *Bathycoccus prasinos* (Eikrem and Throndsen 1990), *Chlorella* sp., and *Nannochloris* sp. (Johnson and Sieburth 1982). We also included some members of the prymnesiophytes (*Imantonia* sp. and CCMP 625) that are supposed to be important contributors to the photosynthetic biomass in open ocean waters. Second, we included strains that have not yet been fully characterized (CCMP 1127, 1192, 1199) and that have been used in previous picoplankton studies, especially that of Hooks et al. (1988). Third, we also included strains that we recently isolated from oligotrophic areas of the tropical Atlantic Ocean (EUM coccoid strains, EUM 8), Sargasso Sea (SARG 71, 72, 73) and Mediterranean Sea (ALMO 1, 2). From TEM preparations (not shown), ALMO strains were identified as *Bathycoccus prasinos*, and EUM 8 and MAX strains were identified as *Pelagomonas* sp. The other tropical Atlantic strains isolated during the *Eumeli* cruise (non-scaly, coccoid cells, EUM 10, 13A, B, 16A, B, 18, and 19) could not be assigned to any described species.

Pigment composition of strains. In the field, the most commonly used technique for identifying the major classes and subclasses in algal assemblages dominated by picoplankton is HPLC pigment analysis. Pigment characterization of cultured strains is therefore essential for further interpretation of HPLC data obtained on natural communities. In our cultured strains, we differentiated six pigment types (Table 2), based on the presence and absence of selected pigments, namely Chl *b*, Chl *c*, prasinoxanthin, lutein, 19′-hexanoyloyfucoxanthin (19′-HF), and 19′-butanoyloxyfucoxanthin (19′-BF) and their ratios to Chl *a*. Groups I, IIA, IIB, and III were previously discriminated by Hooks et al. (1988). Some of the new strains that we analyzed could be included in these groups. In particular, EUM coccoid strains and *Bathycoccus prasinos* were similar in pigmentation to *Micromonas pusilla* (group IIA) (this study and Hooks et al. 1988), *Mantoniella squamata* (Manton et Parke) Desikachery, and a number of unidentified prasinophyte coccoid strains analyzed by either Hooks et al. (1988) or Fawley (1992). *Pycnococcus* spp. strains (CCMP 1192, 1203) and CCMP1199 (group IIB) presented a higher prasinoxanthin to Chl *a* ratio as well as an additional unknown carotenoid similar to that in clone 1201-2 = CCMP 1198 (Hooks

et al. 1988) and *Pseudocourfielda marina* (Throndsen) Manton (Fawley 1992). The question of the taxonomic significance of these two pigment types (IIA vs. IIB) is still not resolved. However, pigment data suggest that unidentified EUM coccoid strains, which we isolated from a number of sites in the tropical Atlantic, have affinities with the Mamiellales (*B. prasinos* and *M. squamata*). Inclusion of these strains into the Mamiellales, which contains nonflagellated species, would support the extension of this order to organisms that lack spider web–like scales, as proposed by Guillard et al. (1991).

In the present study, *Pelagomonas* sp. strains, EUM 8, MAX 71, 72, and 73 had the same major pigments as type III strains in Hooks et al. (1988) and as *Pelagomonas calceolata* (Björnland et al. 1987, Andersen et al. 1993). 19′-BF was the major carotenoid, and 19′-HF was undetectable. However, our Atlantic isolates showed pigment ratios different from that observed in *P. calceolata* (Björnland et al. 1987), the 19′-BF : fucoxanthin ratio being much smaller in the latter species. Further pigment and ultrastructural studies need to be undertaken in order to clarify their taxonomic position, compared to *P. calceolata* (Andersen et al. 1993). CCMP 625 was characterized by the presence of 19′-HF while *Imantonia* sp. contained both 19′-HF and 19′-BF. They were therefore included in separate pigment groups (IV and V, Table 2). While the pigment signature of *Imantonia* sp. resembled that of other prymnesiophytes such as *Phaeocystis* spp. (Buma et al. 1991) and *Imantonia rotunda* (Barlow et al. 1993), CCMP 625, which has only 19′-HF, has an unusual signature among prymnesiophytes.

DNA content and GC% of strains. DNA content may help to further discriminate strains within pigment types at the species level. Accurate measurements of GC% and genome size, which is usually constant in any one species, were performed on isolated nuclei. Genome size and GC% (Table 3, Fig. 1A) were stable within strains of the same species, but large interspecific variations were observed. For example, *Bathycoccus prasinos* strains isolated from different regions of the Mediterranean Sea grouped together (20–22 fg of DNA, GC% = 38.6–39.1), as did all *Pelagomonas* sp. strains isolated from different regions of the Atlantic Ocean (40–41 fg of DNA, GC% = 51.9–52.9). Nucleic DNA content and GC% also clustered together the Atlantic prasinophycean coccoids (EUM coccoids, 19–20 fg of DNA, GC% = 45.4–45.7), suggesting that these strains are a natural genetic assemblage. *Micromonas pusilla, Imantonia* sp., and CCMP 625 differed from these groups in terms of both genome size and base composition. DNA staining was also performed on fixed cells, to include species for which isolation of nuclei was not successful (*Chlorella* sp., *Nannochloris* sp., *Pycnococcus provasolii*, Table 3, Fig. 1). Overall, DNA content and GC% or relative HO and CA3 fluorescence (for fixed cells) provided distinctive characteristics at the species level for almost all the strains analyzed (Table 3, Fig. 1B). For example, the prasinophytes *B. prasinos, M. pusilla,* and the EUM coccoid strains that belonged to group IIA were discriminated. This was not true for *Chlorella* sp. and *Nannochloris* sp., both minute coccoid chlorophyte strains (pigment type I), which were not clearly distinguished. The variability among strains of the same species for Rp_{CA3} and Rp_{HO} was higher than the variability observed for R_{CA3} and R_{HO} measured for isolated nuclei. This was probably due to differences in the penetration of the dyes in the fixed cells. GC% values obtained by this method (Table 3) were also overestimated, probably because of the presence of mitochondrial and chloroplastic DNA.

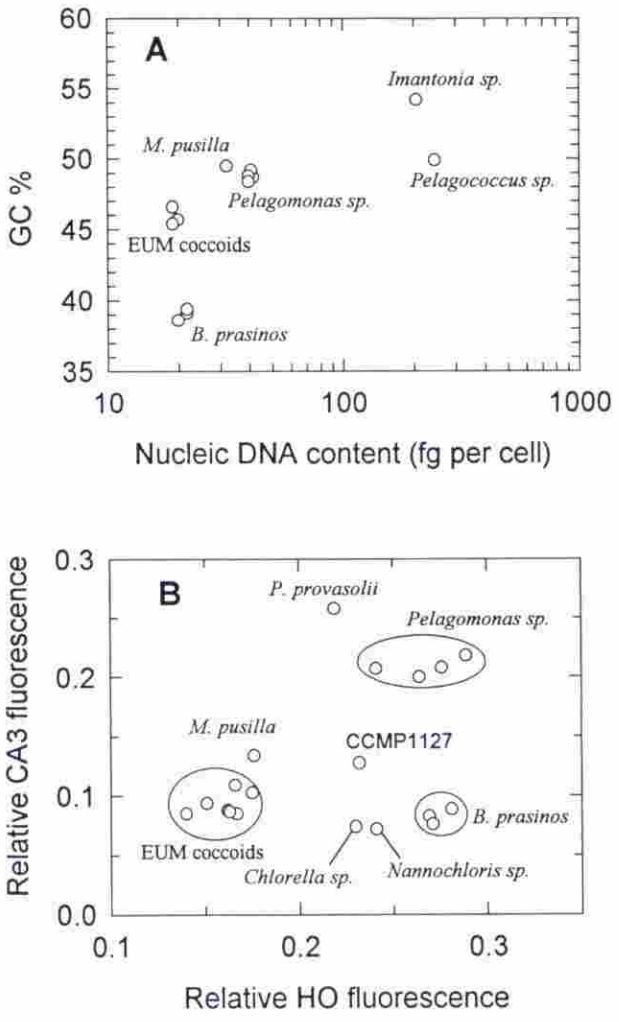

FIG. 1. Discrimination of picoeukaryote species by staining of nuclei or whole cell with DNA-specific dyes. A) Nucleic DNA content (fg) vs. GC% for strains of *Micromonas pusilla* (*M. pusilla*), non-scaly prasinophyte coccoid strains (EUM coccoids), *Pelagomonas* sp. and *Bathycoccus prasinos* (*B. prasinos*). B) Hoechst 33342 vs. chromomycin-relative fluorescence of picoeukaryote strain. *Phaeocystis* sp. (strain PCC 64) was used as internal standard. Note that CCMP 625 and *Imantonia* sp. were not included in this graph for clarity.

However, in contrast to HPLC pigment analysis, this technique gives information on a cell-by-cell ba-

TABLE 3. *Fluorescence ratios of HO, CA3, and PI for picoeukaryotes relative to* Phaeocystis *sp. (PCC 64); nucleic DNA contents and GC% were calculated from these ratios (see Methods). For CCMP 1203 and CCMP 1199, CA3 did not penetrate into the cell. nd = not determined.*

			Fluorescence ratio ± SD					GC%	
	Species	Strains	PI (nuclei)	HO (nuclei)	HO (fixed cells)	CA3 (fixed cells)	DNA (fg)	HO/PI (nuclei)	HO/CA3 (fixed cells)
Chlorophyceae	*Chlorella* sp.	CCMP 253	nd	nd	0.241 ± 0.016	0.072 ± 0.010	nd	nd	44.8
	Nannochloris sp.	CCMP 515	nd	nd	0.230 ± 0.012	0.074 ± 0.011	nd	nd	45.4
	Unidentified	CCMP 1127	nd	nd	0.232 ± 0.013	0.128 ± 0.006	nd	nd	49.6
Prasinophyceae	*Micromonas pusilla*	CCMP 490	0.153 ± 0.004	0.227 ± 0.006	0.176 ± 0.006	0.134 ± 0.009	32	49.5	52.1
	Bathycoccus prasinos	Type strain	0.101 ± 0.006	0.319 ± 0.013	0.281 ± 0.004	0.089 ± 0.005	22	39.1	45.3
	Bathycoccus prasinos	ALMO 1	0.093 ± 0.001	0.304 ± 0.001	0.269 ± 0.024	0.083 ± 0.002	20	38.6	45.1
	Bathycoccus prasinos	ALMO 2	0.101 ± 0.001	0.312 ± 0.007	0.271 ± 0.010	0.076 ± 0.006	22	39.4	44.3
	Unidentified	EUM 10	0.089 ± 0.000	nd	0.162 ± 0.002	0.088 ± 0.013	19	nd	49.4
	Unidentified	EUM 13A	0.092 ± 0.001	0.184	0.167 ± 0.007	0.085 ± 0.005	20	45.7	48.9
	Unidentified	EUM 13B	0.089 ± 0.000	nd	0.175 ± 0.008	0.103 ± 0.001	19	nd	50.0
	Unidentified	EUM 16A	0.088 ± 0.000	0.164 ± 0.009	0.151 ± 0.010	0.094 ± 0.015	19	46.6	50.5
	Unidentified	EUM 16B	0.091 ± 0.002	nd	0.166 ± 0.002	0.109 ± 0.016	19	nd	50.9
	Unidentified	EUM 18	0.090 ± 0.001	0.183 ± 0.008	0.163 ± 0.006	0.087 ± 0.003	19	45.4	49.3
	Unidentified	EUM 19	0.091 ± 0.001	nd	0.140 ± 0.004	0.085 ± 0.003	19	nd	50.3
	Pycnococcus provasolii	CCMP 1203	nd	nd	0.546 ± 0.037	nd	nd	nd	nd
	Pycnococcus sp.	CCMP 1192	nd	nd	0.219 ± 0.009	0.258 ± 0.010	nd	nd	55.2
	Unidentified	CCMP 1199	nd	nd	0.502 ± 0.025	nd	nd	nd	nd
Pelagophyceae	*Pelagomonas* sp.	EUM 8	0.197 ± 0.002	0.312 ± 0.006	0.289 ± 0.008	0.218 ± 0.007	41	48.7	52
	Pelagomonas sp.	MAX 71	0.193 ± 0.006	0.294 ± 0.004	0.276 ± 0.008	0.208 ± 0.021	40	49.2	52
	Pelagomonas sp.	MAX 72	0.190 ± 0.002	0.299 ± 0.004	0.264 ± 0.021	0.200 ± 0.014	40	48.3	51.9
	Pelagomonas sp.	MAX 73	0.186 ± 0.001	0.300 ± 0.004	0.241 ± 0.005	0.207 ± 0.012	40	48.4	52.9
Prymnesiophyceae	Unidentified	CCMP 625	1.167 ± 0.005	1.676 ± 0.015	1.004 ± 0.003	1.137 ± 0.189	245	49.9	54.9
	Imantonia sp.	PCC18561	0.974 ± 0.003	0.955 ± 0.011	0.702 ± 0.030	0.867 ± 0.082	204	54.2	55.5

sis and should provide a way to distinguish and enumerate the main contributors of a mixed community, as long as the assemblage of species is not too complex. DNA content and GC% have not proven to be of phylogenetic or systematic value at higher taxon levels (genus, family, order, or class) among macroalgae (Le Gall et al. 1993), but DNA content appears to be related to cell volume (Shuter et al. 1983, Boucher et al. 1991). It is noteworthy that in comparison to the nuclear DNA levels reported for marine microalgae (100–200, 000 fg DNA·cell^{-1}, Holm-Hansen 1969) or to that of *Arabidopsis thaliana* (166 fg = 1C, Brown et al. 1991), the model system for higher plant genetics, genome sizes encountered for picoeukaryotes are all very small (down to 19 fg·nucleus^{-1}). They are in the range of DNA content reported for prokaryotic organisms (*E. coli*, 4.4 fg, Darnell et al. 1986).

Very little information is available concerning the composition and diversity of natural communities. Are they dominated by a small number of species, or are they very diverse with no dominating taxon? DNA staining with HO and CA3 of preserved natural samples and analysis by flow cytometry provide a way to address this question, as illustrated by the analysis of natural sea water samples collected off Roscoff, in the western English Channel. The community appeared to be homogeneous in terms of cell size and Chl *a* content (light scatters and red fluorescence parameter, Fig. 2A); however, two populations were clearly discriminated in samples stained with either HO or CA3 (Fig. 2B, C), showing that two taxa with different GC% and/or DNA content cooccurred. These taxa (POP 1 and POP 2) contributed 47 and 53%, respectively, of the total community. Similarly, we observed either one, two, or three cooccurring taxa in many oceanic samples of the tropical Atlantic and Mediterranean Sea (unpubl. data). This method may thus be especially useful for rapid and large-scale studies of diversity *in situ*. Nevertheless, this apparently simple situation may not hold for samples in which there may be greater diversity of species, especially for inshore samples. The problem of the identification of the dominating taxa is still unresolved because of the variability of Rp_{CA3} and Rp_{HO} ratios that were obtained for fixed cells. Although results obtained on isolated nuclei show much less variability, the method cannot be used on natural samples because nuclei from photosynthetic picoeukaryotes cannot be distinguished from those of small heterotrophs. It is necessary, therefore, to reduce the variability observed for Rp_{CA3} and Rp_{HO} ratios to be able to use this method for identification of the major taxa in natural populations.

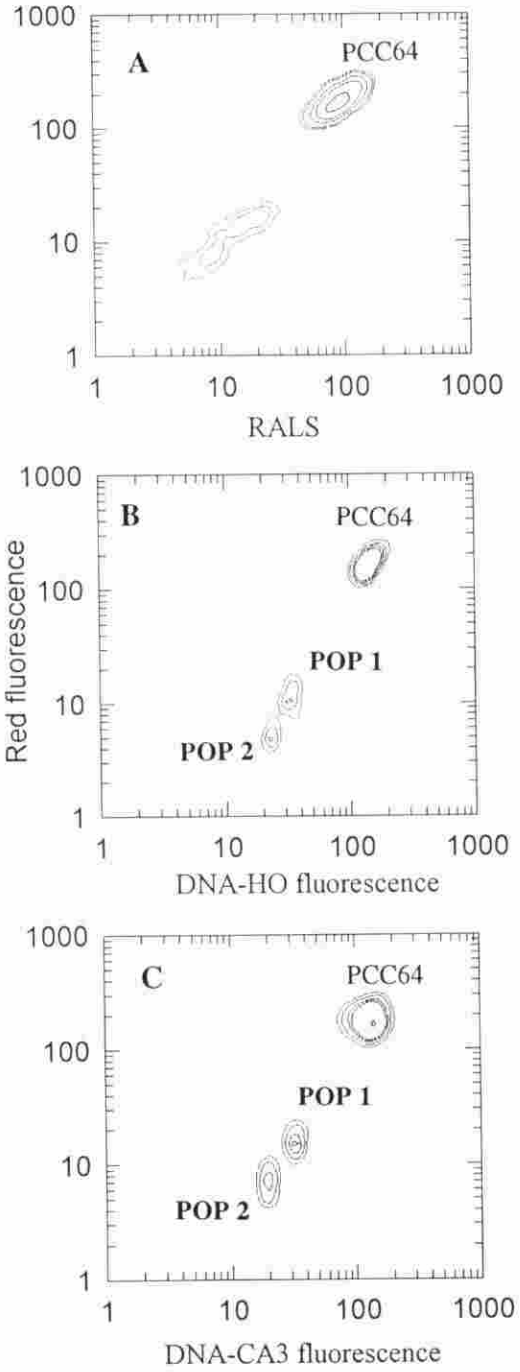

FIG. 2. Contours of cell abundance for a preserved sea water sample collected off Roscoff, as a function of A) Chl fluorescence and RALS, B) fluorescence of Hoechst 33342 and Chl fluorescence, and C) chromomycin and Chl fluorescence. *Phaeocystis* sp. (strains PCC 64) was used as an internal reference. Two populations of picoeukaryotes (POP 1 and POP 2) were discriminated by both dyes.

Volume and Chl a content of strains. Particular attention was given to the measurements of cell equivalent diameters, as size is a criterion for discrimination of picoeukaryote communities. For cultured strains, mean equivalent diameters ranged from 1.2 to 3.5 μm (Table 4). The smallest sizes were observed for a number of chlorophyte strains including *Bathycoccus prasinos* (type strain and newly isolated strains ALMO 1 and 2), the EUM coccoid strains (EUM 13A, 13B, 16A), *Micromonas pusilla*, *Chlorella* sp., and *Nannochloris* sp. Their equivalent diameter hardly exceeded 1.5 μm. Pelagophyte strains (EUM

TABLE 4. *Equivalent diameters and volumes of selected strains, measured with a Coulter counter.*

	Species	Strain	Diameter ± SD (μm)	Volumes (μm³)
Chlorophytes				
Chlorophyceae	*Chlorella* sp.	CCMP 253	1.52 ± 0.17	1.85
	Nannochloris sp.	CCMP 515	1.68 ± 0.20	2.48
Prasinophyceae	*Micromonas pusilla*	CCMP 490	1.53 ± 0.25	1.87
	Bathycoccus prasinos	Type strain	1.57 ± 0.17	2.04
	Bathycoccus prasinos	ALMO 1	1.24 ± 0.15	1.00
	Bathycoccus prasinos	ALMO 2	1.44 ± 0.15	1.57
	Unidentified	EUM 13A	1.33 ± 0.20	1.24
	Unidentified	EUM 13B	1.43 ± 0.18	1.43
	Unidentified	EUM 16A	1.49 ± 0.25	1.49
	Pycnococcus provasolii	CCMP 1203	2.58 ± 0.30	9.03
	Pycnococcus sp.	CCMP 1192	2.19 ± 0.30	5.47
	Unidentified	CCMP 1199	2.53 ± 0.26	8.45
Chromophytes				
Pelagophyceae	*Pelagomonas* sp.	MAX 71	1.99 ± 0.30	4.10
	Pelagomonas sp.	MAX 72	2.01 ± 0.30	4.27
	Pelagomonas sp.	MAX 73	2.07 ± 0.30	4.64
	Pelagomonas sp.	EUM 8	2.09 ± 0.22	4.80
Prymnesiophyceae	Unidentified	CCMP 625	3.06 ± 0.31	15.07
	Imantonia sp.	PCC 18561	3.45 ± 0.40	21.48

8, MAX 71, 72, 73) were slightly bigger, with a 2-μm equivalent diameter. Other picoeukaryote strains (including *Pycnococcus* spp., CCMP625, and *Imantonia* sp.) were all bigger, with diameters exceeding 2.5 μm. We were not able to determine the size of some strains, because of the presence of large numbers of particles in cultures, probably heterotrophic bacteria (EUM 10, 16B, 18, 19, CCMP 1127). Chlorophyll *a* content per cell was highly variable among the species surveyed (2.5–161 fg cell, Table 2), these variations being probably linked to cell volume and light conditions.

Flow cytometric signature of strains. To be able to interpret flow cytometry data that are routinely obtained for natural picoeukaryote communities, relations between scattering properties and cell size as well as between cell red fluorescence and Chl *a* content were investigated (Fig. 3A, B). Light-scattering properties of live cells ($RALS_l$ and $FALS_l$, Table 5) were measured for all the strains (Table 5) and were linearly related to equivalent diameter, D in μm, the highest correlation coefficient being obtained for RALS values.

$$D = 0.13 \times FALS_l + 1.50 \quad (1)$$

$$r^2 = 0.85, \quad P < 0.0005$$

$$D = 1.40 \times RALS_l + 0.97 \quad (2)$$

$$r^2 = 0.95, \quad P < 0.0005$$

Chlorophyll *a* content in fg·cell^{-1} (Table 3) was related to red fluorescence that was measured on live cells (RFL_l) (Table 5) as follows:

$$\text{Chl } a = 0.17 \times RFL_l^{1.5} \quad r^2 = 0.77, P < 0.0005 \quad (3)$$

We then tested the effect of preservation (paraformaldehyde fixation, −80° C storage) that is usually applied for delaying analysis of field samples (Vaulot et al. 1989) on the three parameters measured by flow cytometry (RALS, FALS, and red fluorescence, Table 5). RALS was not significantly modified by fixation (paired *t*- test, $t = 0.167$, $P = 0.869$) in contrast to FALS (*t*-test, $t = 2.362$, $P < 0.05$) and red fluorescence (*t*-test, $t = -3.184$, $P < 0.005$). On average, a 40% decrease of the FALS values was observed for most of the strains after fixation, whereas red fluorescence increased by 25%.

For preserved samples, RALS, FALS, and red fluorescence ($FALS_p$, $RALS_p$, and RFL_p) were related to diameter and Chl *a* content as follows:

$$D = 0.27 \times FALS_p + 1.52 \quad (4)$$

$$r^2 = 0.65, \quad P < 0.0005$$

$$D = 1.21 \times RALS_p + 1.111 \quad (5)$$

$$r^2 = 0.95, \quad P < 0.0005$$

$$\text{Chl } a = 0.22 \times RFL_p^{1.4} \quad (6)$$

$$r^2 = 0.79, \quad P < 0.0005$$

From Equations (5) and (6), mean equivalent diameters and Chl *a* content per cell of natural picoeukaryote communities can be derived from flow cytometry data. Results concerning Chl *a* content per cell provided by Equation (6), however, should only be considered as estimations, because the relationships we observed between Chl *a* content per cell and red fluorescence intensities were derived from different cultured species that are not necessarily representative of the natural communities investigated. Intra-/and interspecific differences in the relationships between cellular Chl *a* and fluorescence per cell, as measured by flow cytometry over a range of irradiances, were observed by Sosik et al. (1989). This variability is largely explained by light-driven differences in the relative abundance of Chl

TABLE 5. *FALS and RALS and red fluorescence of picoeukaryotic strains, measured with flow cytometry. The values are relative RALS, FALS, and red fluorescence of the samples to 0.95-μm fluorescent beads. PF = paraformaldehyde-preserved cells.*

	Species	Strains	FALS		RALS		Red fluorescence	
			Live	PF	Live	PF	Live	PF
Chlorophyceae	*Chlorella* sp.	CCMP 253	1.26	0.93	0.34	0.43	8.52	14.14
	Nannochloris sp.	CCMP 515	1.56	1.33	0.39	0.41	10.92	13.36
	Unidentified	CCMP 1127	2.09	1.58	0.49	0.49	11.30	13.02
Prasinophyceae	*Micromonas pusilla*	CCMP 490	1.03	0.73	0.51	0.41	11.72	16.96
	Bathycoccus prasinos	Type strain	1.16	0.58	0.41	0.36	11.50	14.93
	Bathycoccus prasinos	ALMO 1	0.63	—	0.29	—	7.36	—
	Bathycoccus prasinos	AMLO 2	0.74	0.42	0.32	0.28	9.95	13.08
	Unidentified	EUM 10	0.38	0.31	0.37	0.33	11.65	12.49
	Unidentified	EUM 13A	0.37	0.23	0.34	0.29	10.10	12.36
	Unidentified	EUM 13B	0.39	0.27	0.40	0.32	11.16	12.59
	Unidentified	EUM 16A	0.33	0.21	0.37	0.28	10.12	12.14
	Unidentified	EUM 16B	0.46	0.27	0.38	0.35	13.07	15.16
	Unidentified	EUM 18	0.46	0.31	0.36	0.29	10.67	12.67
	Unidentified	EUM 19	0.42	0.29	0.35	0.33	11.48	12.3
	Pycnococcus provasolii	CCMP 1203	5.30	4.22	1.28	1.34	37.43	49.44
	Pycnococcus sp.	CCMP 1192	4.25	4.59	0.80	1.10	29.54	24.71
	Unidentified	CCMP 1199	5.39	4.21	1.20	1.21	34.80	43.44
Pelagophyceae	*Pelagomonas* sp.	EUM 8	2.27	0.78	0.68	0.62	18.10	18.58
	Pelagomonas sp.	MAX 71	3.01	0.87	0.71	0.62	27.02	40.56
	Pelagomonas sp.	MAX 72	2.98	0.82	0.69	0.67	27.82	37.55
	Pelagomonas sp.	MAX 73	2.64	0.84	0.72	0.68	30.84	34.73
Prymnesiophyceae	Unidentified	CCMP 625	16.01	5.95	1.62	1.57	66.89	89.76
	Imantonia sp.	PCC 18561	14.43	3.29	1.61	1.90	59.77	97.62

a and accessory pigments that absorb light and transfer energy to Chl *a*, thus enhancing fluorescence signals.

Interpretation of flow cytometric data for natural picoeukaryote communities. Flow cytometric data obtained from different oceanic provinces invariably show a well-defined population of unnamed small eukaryotic algae (1–3 μm in diameter) (Li and Wood 1988, Li 1989, Vaulot et al. 1990, Li et al. 1992, Campbell and Vaulot 1993). Using Equations (5) and (6), RALS and red fluorescence measured by flow cytometry provided estimates of cell size and Chl *a* content of natural picoeukaryote communities originating from a variety of oligotrophic areas of the North Pacific, northeastern Atlantic, northwestern Mediterranean, and Alboran Sea (Table 6). Mean cell size of the communities did not vary much between the different sites, ranging from 1.3 to 2 μm, in contrast to Chl *a* content, which fell in a threefold range, from 6 to 16 fg·cell^{-1}.

The amplitude of variation of cellular Chl *a* could be rather high within a single area. For the northeastern tropical Atlantic area investigated during the EUMELI 4 cruise, Chl *a* content per cell ranged from 0.4 to 70 fg·cell^{-1} (Table 6). Such a wide range is accounted for by photoacclimation (Claustre and Gostan 1987) that leads to an increase in red fluorescence with depth (Campbell and Vaulot 1993). However, Chl *a* content is highly dependent on light irradiance (Claustre and Gostan 1987, Sosik et al. 1989). As already stated, for computation of Chl *a* biomass from flow cytometry measurements, it would be more accurate to establish relationships for species that are representative of natural communities.

CONCLUSION

How are the picoeukaryote strains available in culture representative of natural populations? Organisms containing Chl *b*, 19′-BF, and 19′-HF seem to dominate the phytoplankton community in the euphotic zone of the open North Atlantic (Gieskes and Kraay 1986, Williams and Claustre 1991, Barlow et al. 1993), the West Equatorial and North Pacific (Everitt et al. 1990, Ondrusek et al. 1991, Letelier et al. 1993), and the Red Sea (Veldhuis and Kraay 1993). Moreover, pigment fingerprints suggest dominance of Chl *b*, 19′-HF, and 19′-BF at the depth of the deep Chl maximum, where 1–3 or 1–5-μm organisms dominated the Chl *a* biomass (northeastern Atlantic [Gieskes and Kraay 1986, Williams and Claustre 1991] and Central North Pacific [Letelier et al. 1993]). Sources of these pigments *in situ* are supposed to be prymnesiophytes, chrysophytes, and "green algae."

Chlorophyll *c* containing algae, *Emiliana huxleyi*, *Phaeocystis* spp., *Imantonia rotunda*, *Chrysochromulina* spp., and *Pelagococcus subviridis* (3–5-μm species), are suspected of contributing to the chromophyte community *in situ* because they contain 19′-BF and/or 19′-HF and because they were isolated or observed in many stations of the world ocean (Everitt et al. 1990, Barlow et al. 1993). However, these species are larger in size than natural picoeukaryote populations (1.3–2 μm in diameter, Table 6) observed by flow cytometry in open subtropical regions. *Imantonia* sp. (PCC 18561) and CCMP 625 are also larger (Table 4). The only chromophyte in culture with a size similar to that of field populations is *Pelagomonas* sp. In addition, *Pelagomonas* sp. strains have 19′-BF

TABLE 6. *Equivalent diameters and Chl* a *content for natural populations of picoeukaryotes, calculated from flow cytometry data (RALS and red fluorescence) using Equations (4) and (6). n = number of samples analyzed.*

Area	Area coordinate	Program, cruise	Date	Samples		Equivalent diameter (μm)			Chl *a* (pg·cell^{-1})			
				n	Fixed or live	Min	Max	Mean	Min	Max	Mean	Source[a]
NE Atlantic	36° N, 15° W	JGOFS, *MedAtlante*	Aug 1989	12	Fixed	1.46	2.69	1.79	4.03	33.85	15.42	1
NE Atlantic	20–21° N, 31–32° W	JGOFS, *Eumeli* 4	Jun 1992	162	Fixed	1.20	2.26	1.35	0.37	70.06	13.07	2
N Pacific	22° N, 158° W	JGOFS, *HOT*	1991	44	Fixed	1.54	3.07	2.03	1.14	43.69	12.30	5
NW Mediterranean Sea	40–43° N, 4–6° E	EROS 1, *Discovery* #179	Jan 1989	62	Live	1.28	2.06	1.62	3.37	13.77	6.92	3
NW Mediterranean Sea	40–43° N, 4–6° E	EROS 1, *Bannock*	Jul 1989	80	Fixed	1.47	2.53	1.82	2.17	41.63	7.50	4
W Mediterranean Sea (Alboran Sea)	36° N, 5° W	EROS II, *Valdivia* #121	Mar 1992	102	Fixed	1.42	1.97	1.59	3.37	17.28	9.75	6

[a] 1 = Vaulot, Partensky, and Claustre, unpubl.; 2 = Partensky and Blanchot, unpubl.; 3 = Vaulot and Partensky, unpubl.; 4 = Vaulot and Partensky (1992); 5 = Campbell and Vaulot (1993); 6 = Vaulot and Marie (1993).

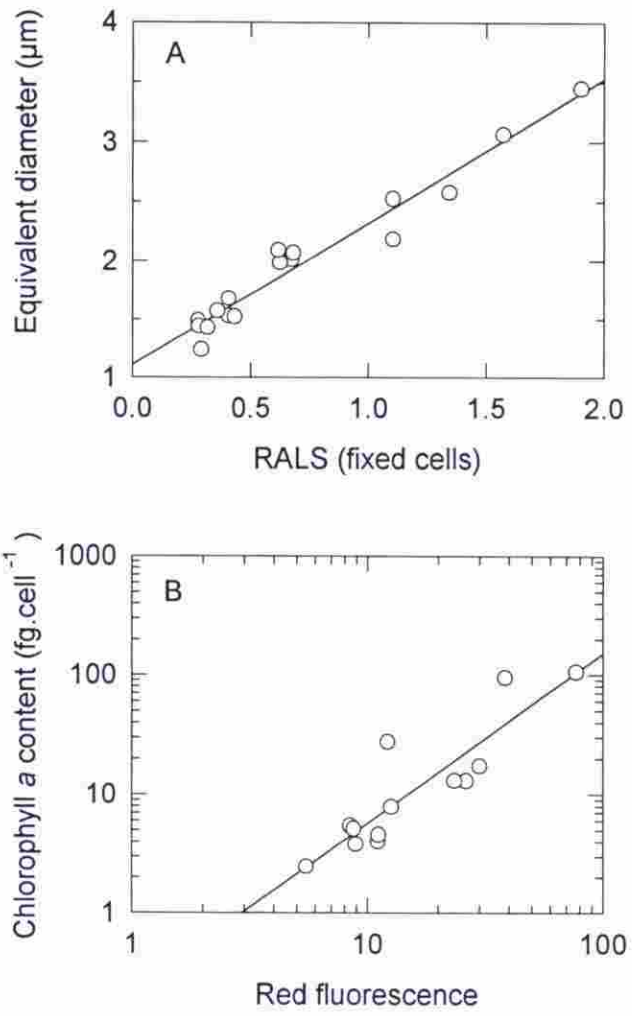

FIG. 3. Calibration of RALS and red fluorescence measured for fixed picoeukaryotes with, respectively, diameter of live cell and Chl *a* content. A) RALS (in arbitrary units) vs. diameter of picoeukaryotic strains. B) Red fluorescence measured with flow cytometry (arbitrary units) vs. Chl *a* content (fg·cell^{-1}). See text for equations of regression lines.

as the major carotenoid, and their fucoxanthin : 19′-BF ratios (0.05–0.14, Table 4) and 19′-BF : Chl *a* ratios (0.65–1.47, Table 4) measured for cultures at low blue light match those (0.08 and 1.60, respectively) calculated by Everitt et al. (1990) for deep populations of 19′-BF-containing organisms in the western equatorial Pacific. Among the cultured picoeukaryotes that we surveyed, *Pelagomonas* sp. strains are therefore the most representative of natural picoplanktonic communities in subtropical waters. Additional support for this view comes from the fact that the strains isolated in culture come from very distinct locations (the type species *P. calceolata* was isolated from the Pacific Ocean, and our strains were isolated from the Sargasso Sea and the northeastern Atlantic), suggesting a worldwide distribution. More recently, we also isolated a strain from the Red Sea (M. Veldhuis and N. Simon, unpubl. data). Attention should also be paid to prymnesio-

phyte strains such as CCMP 625 because a high 19′-HF : Chl *a* ratio was detected in this strain. However, one may expect that further studies on monoalgal cultures will reveal a wider systematic distribution pattern of 19′-HF and 19′-BF, especially in cells of very small size, that were often overlooked in microscopic analyses.

Algae contributing to the Chl *b* biomass are hypothesized as belonging either to the chlorophytes or to the prasinophytes. Prasinophytes could be both prasinoxanthin-containing and-lacking species since occasional occurrence of significant amounts of prasinoxanthin was reported in the tropical Atlantic and open Pacific by Hooks et al. (1988), Everitt et al. (1990), and Letelier et al. (1993). *Micromonas pusilla, Bathycoccus prasinos, Nannochloris*-like and *Chlorella*-like organisms, and *Pycnococcus provasolii* (1–3 μm in diameter) were reported from a number of sites in the Atlantic and/or Pacific Oceans (Johnson and Sieburth 1982, Murphy and Haugen 1985, Eikrem and Throndsen 1990, Guillard et al. 1991, Campbell et al. 1994b). The non-scaly, prasinoxanthin-containing coccoid strains that we isolated from several sites in the tropical Atlantic (EUM coccoids) might also contribute to the Chl *b* and prasinoxanthin biomass *in situ*. However, strains brought into culture provide a biased sample of the natural flora. As recently stated by Letelier et al. (1993), prasinoxanthin, violaxanthin, and lutein, markers for chlorophytes, were usually only present in low amounts or even undetectable in most of the open ocean areas that were investigated using HPLC (Everitt et al. 1990, Williams and Claustre 1991, Letelier et al. 1993). There is no report of a eukaryotic species in which pigmentation would explain the combined presence of high amounts of Chl *b* and low amounts of prasinoxanthin, lutein, and violaxanthin *in situ*. It is noteworthy that the recently discovered prokaryotic genus *Prochlorococcus* (Prochlorophyta) (Chisholm et al. 1992) contains divinyl Chl *b* (Chl b_2), which until recently was not separated from "normal" monovinyl Chl *b* (Chl b^1) by reverse-phase HPLC. The presence, in field samples, of this divinyl form is now taken into account by taxonomic algorithms designed to assess the contribution of algal groups from pigment ratios to Chl *a* (Everitt et al. 1990, Barlow et al. 1993, Letelier et al. 1993). Only recently could Chl b_2 be separated from Chl b_1 by reverse-phase HPLC (Goericke and Repeta 1993). Still, the presence of Chl b_1 is not a good tracer of eukaryotic taxa, since under certain conditions *Prochlorococcus* can synthesize Chl b_1 (Partensky et al. 1993, Moore et al. 1994). *Prochlorococcus*, which occurs at high concentration in the euphotic zone of oligotrophic oceans and seas (Chisholm et al. 1988, Olson et al. 1990a, Vaulot et al. 1990), might explain in part the large amount of Chl *b* reported for these oceanic regions.

In conclusion, only *Pelagomonas* strains seem to be really representative of open oligotrophic areas. All the other strains brought into culture are obviously present in these areas but most likely at insignificant concentrations. This demonstrates the huge amount of work still needed to characterize eukaryotic picoplankton. The combination of flow cytometry with HPLC pigment analysis is indeed useful in that respect. However, new approaches, such as immunolabeling, or the design of taxon-specific ribosomal RNA probes (Amann et al. 1990), are probably needed to take quantum steps in solving the true nature of picoeukaryote communities.

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BUOYANCY REGULATION IN THE COLONIAL DIAZOTROPHIC CYANOBACTERIUM *TRICHODESMIUM TENUE*: ULTRASTRUCTURE AND STORAGE OF CARBOHYDRATE, POLYPHOSPHATE, AND NITROGEN[1]

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ABSTRACT

Trichodesmium tenue *Wille (1904) was examined using transmission electron microscopy to determine the role of carbohydrate, phosphorus, and nitrogen storage in buoyancy regulation. Carbohydrate storage area (mean = 2.06 ± 0.61 [SE] μm²; 6.62% of total cell area) in negatively buoyant colonies (NBCs) was significantly higher (P < 0.001) than in positively buoyant colonies (PBCs) (mean = 0.38 ± 0.06 μm²; 0.73%). Distinct diel periodicity of carbohydrate content was found in NBCs demonstrated by an increase from darkness to afternoon. Polyphosphate content was significantly higher (P < 0.001) in NBCs, with a mean of 0.44 ± 0.10 μm² (1.54%), as compared to PBCs, with a mean of 0.14 ± 0.05 μm² (0.24%). Polyphosphate content increased in NBCs from morning to evening, and PBCs had a 10% decrease from morning to afternoon. Calculations indicated that averaged effects of polyphosphate on increased cell density is approximately 20% of that from carbohydrate accumulation. Density contribution due to ballast weight of carbohydrate and polyphosphate indicated that NBCs were 12 times more dense than PBCs. Mean area of cyanophycin granules (N storage) was not significantly different between PBCs and NBCs. In conclusion,* Trichodesmium tenue *can regulate buoyancy by carbohydrate ballasting similar to that noted in limnetic cyanobacteria. Polyphosphate storage and possibly nitrogen storage products play a significant role in buoyancy regulation.*

Key index woods: buoyancy; carbohydrate ballasting; cyanobacteria; polyphosphate; Trichodesmium tenue; *vertical migration*

Buoyancy regulation in limnetic cyanobacteria is well studied. Several mechanisms of buoyancy regulation for depth-keeping or vertical migration have been observed, and these include the "dilution" of gas vesicles by turgor pressure collapse, cell growth, or carbohydrate ballasting. (Walsby 1972, van Rijn and Shilo 1985, Kromkamp and Konopka 1986). Other factors shown to affect buoyancy are avail-

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