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Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA)

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Abstract—The structure of the picoplankton community in the subtropical Pacific was examined on four depth profiles, one from each season, sampled at the Hawaii Ocean Time-series station ALOHA (22°45′N, 158°W). Three cell populations were discriminated by flow cytometry: *Prochlorococcus* prochlorophytes, *Synechococcus* cyanobacteria, and picoeukaryotes. *Prochlorococcus* were the most abundant component (maximum *ca* 2×10^5 cells ml^{-1}). Unlike previous reports, their concentration was almost constant down to roughly 100 m, with a slight maximum at the surface or near the chlorophyll maximum. Cellular chlorophyll fluorescence increased 50-fold between surface and deep populations. One distinguishing feature of the community off Hawaii was the co-occurrence near the chlorophyll maximum of at least two distinct *Prochlorococcus* populations with different chlorophyll and DNA contents. Throughout the year, *Synechococcus* abundance was two orders of magnitude lower and there was no seasonal alternation between *Prochlorococcus* and *Synechococcus*, as observed in the northern Sargasso Sea. *Synechococcus* populations did not extend below 120 m and were dominated by high phycourobilin cell types. Picoeukaryote abundance was quite similar to that of *Synechococcus*, but these cells extended deeper in the water column. Their chlorophyll fluorescence exhibited much less depth variation than *Prochlorococcus* or *Synechococcus*. Seasonal variability was small (<2- to 3-fold) for all three components of the picoplankton, not only for cell abundance but also for cellular parameters such as light scatter or pigment fluorescence. *Synechococcus* populations exhibited the largest seasonal changes (e.g. abundance maximum and chlorophyll fluorescence varied 3-fold). Picoplankton community structure in the Pacific Ocean appears to be distinct from previous reports for other areas. In comparing station ALOHA to the Atlantic Ocean (especially the Sargasso Sea) and the Mediterranean Sea, depth-integrated abundances of *Prochlorococcus* were higher, that of *Synechococcus* were lower, and that of picoeukaryotes were similar. We believe this structure, dominated by *Prochlorococcus*, may be typical for subtropical open-ocean regions.

INTRODUCTION

IT is now widely recognized that the smaller size classes of oceanic plankton, and especially the picoplankton <2 μm, are important contributors to both photosynthetic biomass and production, particularly in tropical oligotrophic regions (e.g. for the Pacific Ocean: BEERS *et al.*, 1975; LI *et al.*, 1983; CHAVEZ, 1989; LE BOUTEILLER *et al.*, 1992). For example, BIENFANG *et al.* (1984) showed that around Hawaii as much as 80% of the chlorophyll (Chl) *a* biomass could be due to cells <3 μm. TAGUCHI and LAWS (1988), going one step further, showed that up to 35% of the cells <2 μm could pass through a GF/F filter.

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Initially, the ecology of the picoplankton was investigated using either electron microscopy (JOHNSON and SIEBURTH, 1982) or epifluorescence microscopy (MURPHY and HAUGEN, 1985); however, neither method is able to provide quantitative data with high frequency sampling. The routine use of flow cytometry to collect quantitative data for abundances and fluorescence properties of oceanic phytoplankton populations (OLSON *et al.*, 1985) has made it possible to obtain a far more accurate picture of the picophytoplankton community. In particular it has led to the discovery of *Prochlorococcus* (CHISHOLM *et al.*, 1988, 1992).

Most of the recent (i.e. following the discovery of *Prochlorococcus*) studies on the composition of the picoplankton have been conducted in the North West Atlantic Ocean (LI and WOOD 1988; NEVEUX *et al.*, 1989; OLSON *et al.*, 1990a), the North East Atlantic Ocean (VELDHUIS and KRAAY, 1990) and the Mediterranean Sea (VAULOT *et al.*, 1990), but few data are available for the Pacific. In the Pacific, TAKAHASHI and HORI (1984) first reported pictures of prokaryotic cells in the deep chlorophyll maximum layer (DCML) that possessed closely appressed thylakoids, a characteristic of *Prochlorococcus* (CHISHOLM *et al.*, 1988). CHISHOLM *et al.* (1988) detected *Prochlorococcus* off the California coast. Divinyl Chl *a*, a pigment marker of *Prochlorococcus*, has been reported from the Banda Sea (GIESKES *et al.*, 1988) and from the central tropical Pacific (ONDRUSEK *et al.*, 1991). Finally, CHAVEZ *et al.* (1991) directly recorded *Prochlorococcus* by flow cytometry at the Equator. All these data plead for a detailed study of Pacific picoplankton that would include *Prochlorococcus*.

The Hawaii Ocean Time-series at station ALOHA (A Long-term Oligotrophic Habitat Assessment) is a 5-year US-GOFS (Global Ocean Flux Study) project that began in October 1988 to identify and quantify the processes that control biogeochemical cycling in the subtropical central North Pacific Ocean (KARL and WINN, 1991). Thus, it is important to have an accurate description of the community structure to elaborate our current models of the food web in the oligotrophic ocean. Here we present data obtained by flow cytometry on the picophytoplankton community structure at station ALOHA during 1991. We focus on four cruises, one during each of the four seasons, and compare the typical community structure of the subtropical North Pacific to that reported in the North Atlantic Ocean and the Mediterranean Sea.

MATERIALS AND METHODS

Station location

The Hawaii Ocean Time-series (HOT) station ALOHA (Fig. 1) is located about 100 km north of Oahu, Hawaii (22°45′N, 158°W). Station ALOHA is a deep-water open-ocean site characterized by a seasonally variable mixed layer (WINN *et al.*, 1991). The surface mixed layer was shallower during the winter and spring cruises, and deeper during the summer and fall cruises (Fig. 2). Surface water temperatures are relatively warm and ranged from 23°C in winter up to 26°C in summer. Chlorophyll profiles (stimulated *in situ* fluorescence) were measured by the JGOFS core program using a flash fluorometer (Sea Tech, Corvallis, OR) (WINN *et al.*, 1993). The depth of the DCML varied between 105 m and 125 m among the four cruises (Fig. 2). The average depth of the DCML at station ALOHA for the last 3 years was approximately 112 m, and the incident light level at the DCML ranged from 0.08 to 3% of surface incident PAR (LETELIER *et al.*, in press). Both

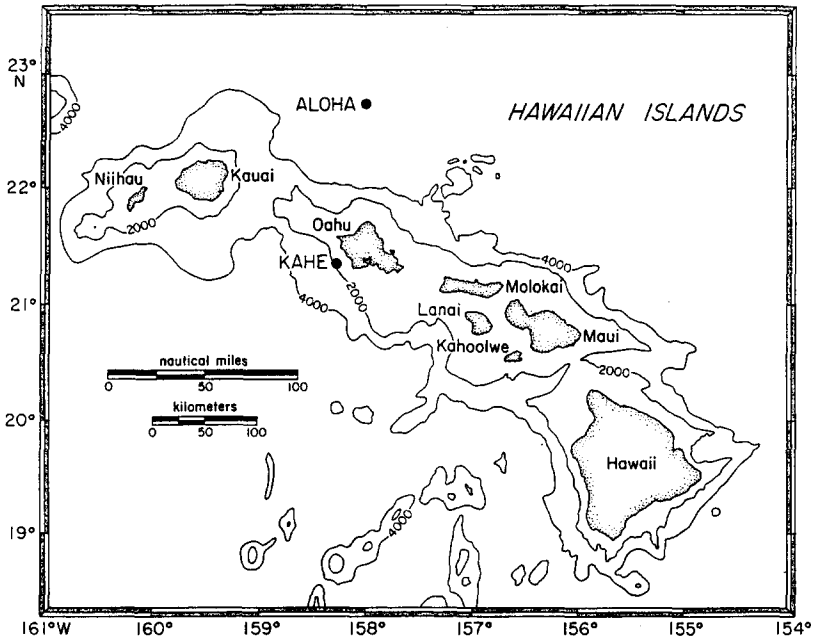

Fig. 1. Location of station ALOHA, the Hawaii Ocean Time-series station where samples were collected, 100 km north of Oahu, Hawaii.

nitrate and ammonia nutrient levels were below the level of detection within the euphotic zone, and typically the top of the nitracline occurred between 100 and 200 m (WINN *et al.*, 1991, 1993). We defined the nitracline as the first appearance of 0.1 μM NO_3 + NO_2, and, in general, the nitracline occurred just below the DCML (except during the spring cruise; Fig. 2).

Sample collection and preservation

Monthly samples were collected from 10–12 depths between 0 and 200 m using a Niskin rosette sampler. Sample depths on each cruise corresponded approximately to 46, 23, 9, 2, 0.85, 0.24, 0.08 and 0.03% of the surface incident light level, with additional samples taken around the depth of the DCML. Replicate 1 ml volumes were preserved, and after 10 min frozen quickly in liquid nitrogen and stored at −70°C until analysis following VAULOT *et al.*'s (1989) method, except that paraformaldehyde (0.2% final concentration) was used instead of glutaraldehyde. We have selected profiles from cruises HOT-23 (1–6 February 1991), HOT-25 (8–12 April 1991), HOT-28 (8–12 July 1991), and HOT-31 (19–24 October 1991), one for each season.

Flow cytometry

All data were collected using a Coulter (Hialeah, FL) EPICS 753 flow cytometer equipped with two 5 W Argon lasers and an automatic sampling device (MSDS). Both a confocal lens and a Biosense flow cell were used to increase sensitivity (OLSON *et al.*,

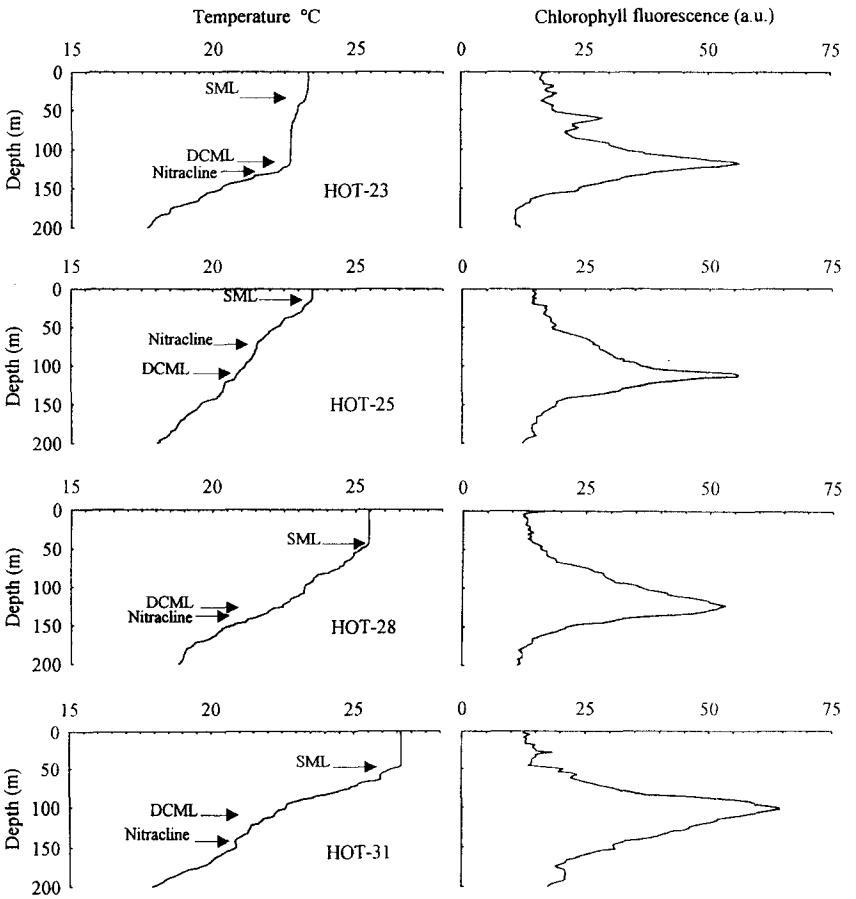

Fig. 2. CTD temperature and *in situ* fluorescence (arbitrary units) profiles for HOT-23 (Feb), HOT-25 (Apr), HOT-28 (Jul), and HOT-31 (Oct) casts from which samples were collected. Depths of the surface mixed layer (SML), deep chlorophyll maximum layer (DCML), and the top of the nitracline (defined as the first appearance of 0.1 μM NO_2 + NO_3) are marked for each cruise. Ammonia concentrations were not determined.

1990a). The laser and filter set-ups were very similar to those used by OLSON *et al.* (1990a) and VAULOT *et al.* (1990) and are given in Table 1. Three different populations were discriminated on the basis of right angle light scatter (RALS) and pigment fluorescence as described in VAULOT *et al.* (1990): *Prochlorococcus*, *Synechococcus*, and small eukaryotic algae. Most of the measurements were made at an excitation wavelength of 488 nm (Table 1); however, *Prochlorococcus* in the surface layer frequently were too dim to be recorded accurately with this set-up. To increase sensitivity, we therefore had to use all lines (457–515 nm, OLSON *et al.*, 1990a), which provided 4 W of power and allowed us to detect *Prochlorococcus* up to the surface. Samples were spiked with 0.57 μm Polysciences (Warrington, PA) Fluoresbrite standard beads (same batch used by OLSON *et al.*, 1990a, b). To investigate the pigment composition of *Prochlorococcus*, some samples were run using the 457 nm line which preferentially excites Chl *a* in contrast to 488 nm which preferentially excites Chl *b*. To examine the DNA content of *Prochlorococcus*, some

Table 1. *Flow cytometry analyses: set-ups, collected parameters and their interpretation. A + sign in a given column means that the corresponding population has been analysed with the set-up. N.D.: not determined. PE: phycoerythrin. Chl: chlorophyll*

Laser line (nm)	488	457–515 (All lines)	457	488/UV
Power (W)	1	4	0.2	1/0.2
Sample volume (ml)	0.3	0.1	N.D.	N.D.
Parameters collected (filter cut-offs):				
Right angle light scatter	Size*	Size*	Size*	Size*
Blue fluorescence (450 nm ± 40 d.f.)				DNA
Green fluorescence (525 nm ± 40 d.f.)	PE			
Orange fluorescence (575 nm ± 40 d.f.)	PE	PE	PE	
Red fluorescence (680 nm ± 40 d.f.)	Chl	Chl	Chl	Chl
Populations analysed:				
Prochlorococcus	+†	+	+†	+†
Synechococcus	+	+		
Picoeukaryotes	+		+	

*Right angle light scatter is actually a complex function of size and refractive index (Morel, 1991).

†*Prochlorococcus* could not be detected accurately in the surface mixed layer with this set-up.

samples were stained with 2 μg ml^{-1} of DAPI (Sigma, St Louis, MI) and analysed with dual UV/488 nm excitation as described in Boucher *et al.* (1991).

Data were collected in list mode, transferred to a personal computer and analysed using the CYTOPC software (Vaulot, 1989). All signals were normalized to that of the 0.57 μm beads. A T-test (SYSTAT; Evanston, IL) was used to test significance of DNA content between *Prochlorococcus* populations.

RESULTS

Prochlorococcus

Prochlorococcus were extremely abundant (up to 2 × 10^5 cell ml^{-1}) at station ALOHA throughout the year. The vertical structure of *Prochlorococcus* abundance was quite similar in all profiles (Fig. 3A). Cell numbers were highest in the upper 100 m. In some cases peak abundance occurred at the surface (Table 2), but often was located just above the DCML, followed by an exponential decrease below (Fig. 3A). *Prochlorococcus* were still observed, albeit at low concentrations, at 200 m. The peak magnitude varied at most by a factor of 2 seasonally. Chl fluorescence per cell increased 50-fold between its minimum in the surface mixed layer and its maximum below the DCML (Fig. 3B). Chl fluorescence was quite constant throughout the year both in the surface mixed layer (0.17–0.28) and below the DCML (8.5–10), but was much more variable in the region extending between the bottom of the surface layer and the top of the nitracline. The ratio of Chl fluorescence excited at 457 and 488 nm (Table 1), which covaries with the cellular Chl *b*:*a* ratio, increased almost 3-fold between the surface layer and 175 m in the profile collected

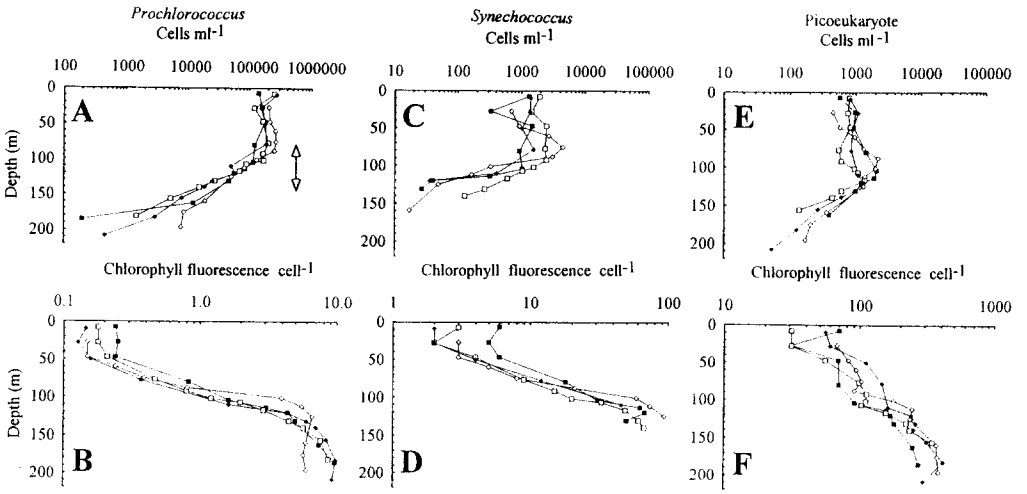

Fig. 3. Depth profiles for HOT-23 (■), HOT-25 (□), HOT-28 (◆), and HOT-31 (◇) of (A) *Prochlorococcus* abundance: range of depths where two distinct populations co-occurred is indicated by double-headed arrow: HOT-23 (80–114 m); HOT-25 (102–140 m); HOT-28 (110–120 m); HOT-31 (88–112 m); (B) *Prochlorococcus* chlorophyll fluorescence cell^{-1} (all lines excitation); (C) *Synechococcus* abundance; (D) *Synechococcus* chlorophyll fluorescence cell^{-1} (488 nm excitation); (E) picoeukaryotic algae abundance; (F) picoeukaryote chlorophyll fluorescence cell^{-1} (488 nm excitation). All fluorescence measurements were normalized to 0.57 μm YG (Polysciences) beads.

Table 2. Cell concentrations and depths of the abundance maxima for vertical profiles of Prochlorococcus, Synechococcus *and picoeukaryotes*

Population	Cruise	Cell concentration (10^3 cell ml^{-1})	Depth of maximum (m)
Prochlorococcus	HOT-23	176	47
	HOT-25	240	8
	HOT-28	272	9
	HOT-31	266	75
Synechococcus	HOT-23	1.48	47
	HOT-25	2.45	47–92
	HOT-28	1.56	78
	HOT-31	4.44	75
Picoeukaryotes	HOT-23	2.10	104
	HOT-25	1.37	117
	HOT-28	1.34	120
	HOT-31	2.19	101

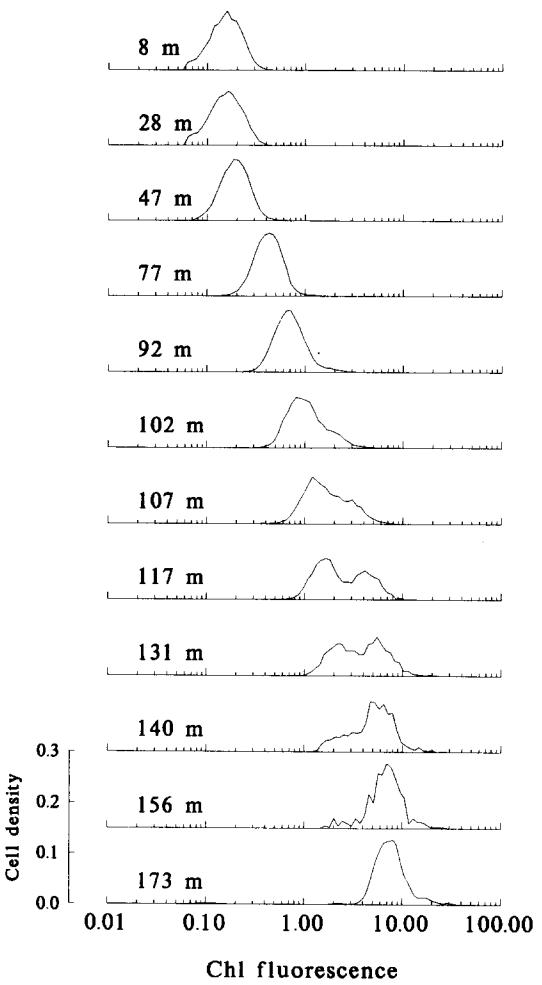

Fig. 4. Bimodal *Prochlorococcus* Chl fluorescence distributions are a permanent feature observed in all samples. Cruise HOT-25 is a typical example. *X* axis: Chl fluorescence normalized to 0.57 μm beads. *Y* axis: relative cell density. While the distributions are unimodal from the surface down to 90 m as well as below 150 m, between 100 and 140 m two peaks are observed with a 3-fold difference in fluorescence.

in October (2 to 5.5). HPLC analyses of divinyl Chl *a* and *b* for this same profile showed the identical trend: a several-fold increase with depth (CAMPBELL *et al.*, 1992; BIDIGARE, unpublished).

One permanent feature observed for all cruises was the presence of bimodal *Prochlorococcus* populations characterized by different RALS and Chl fluorescences (Fig. 4). Such bimodal populations were observed around the DCML between 80 and 140 m within a depth interval that was wider in winter and spring than in summer and fall (Fig. 3). The mode fluorescence of the low Chl population (termed dim, to follow OLSON *et al.*, 1988) was about 2–3 times smaller than that of the high Chl (bright) one (Fig. 4). The dimmer cells also were probably smaller as reflected by the lower RALS, which is a function of size

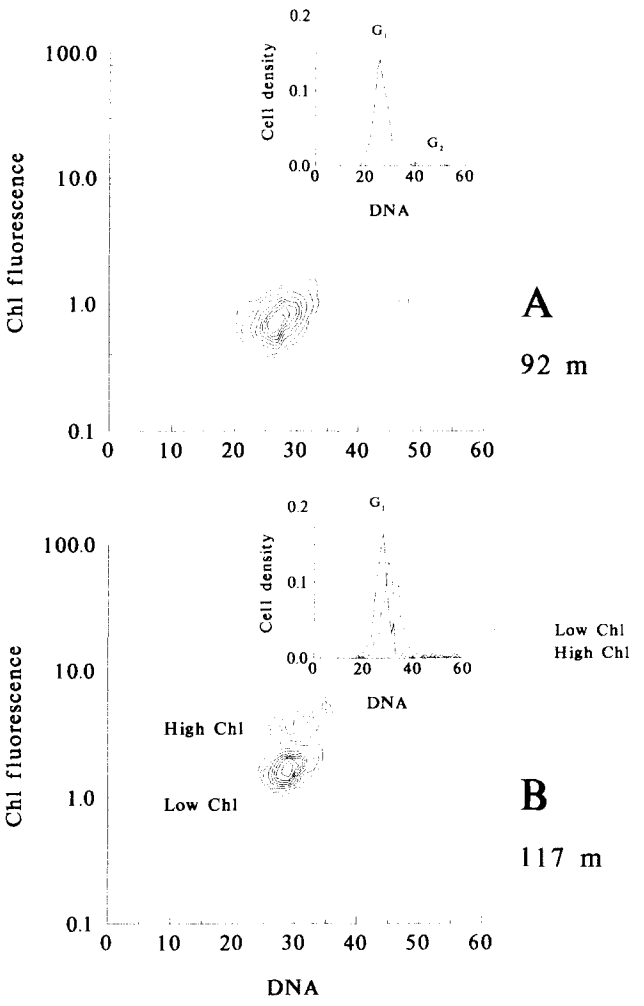

Fig. 5. *Prochlorococcus* cytograms of DNA (stained with DAPI, linear scale, arbitrary units) versus Chl fluorescence (log scale, normalized to 0.57 μm beads) at two depths 92 m (A) and 117 m (B) for HOT-25. The insets correspond to the one-parameter DNA distributions. At 117 m, the high and low Chl populations were gated and their DNA distributions obtained separately.

(data not shown). Using the April data as an example, the relative proportion of the brighter population increased from a few per cent at 92 m to 100% at 156 m (Fig. 4). Although the increase in the average Chl fluorescence for the total population (i.e. dim plus bright cells) between these depths (Fig. 3B) could be attributed to the increased proportion of bright cells, we found the modal fluorescence of both the dim and the bright cells increased independently (Fig. 4). To probe further the nature of these two populations, some samples were stained with DAPI and analysed with flow cytometry (BOUCHER *et al.*, 1991) to assess cell DNA content (Fig. 5). At 92 m (Fig. 5A), where a single population was visible on the Chl distribution, the DNA histogram revealed a characteristic pattern with both a major and a minor peak; although these are prokaryotic

Table 3. Synechococcus *pigments indexes for different strains and samples at station ALOHA. For HOT isolates, isolation date/sample depth are given in parentheses*

Strain/sample	Emission ratio 525:575	Excitation ratio 488:457–515
WH7805 (PEB only)	0.002	1.06
WH7803 (low PUB:PEB)	0.018	1.24
WH8103 (high PUB:PEB)	0.037	1.83
WH8107 (high PUB:PEB)	0.039	2.06
Station ALOHA: mean (n=34)	0.032	2.19
Station ALOHA: range	0.023–0.057	1.5–2.6
HOT9101 (May 91; 135 m)	0.057	1.96
HOT9103 (June 91; 130 m)	0.059	2.02
HOT9104 (Oct 91; 5m)	0.053	2.22
HOT9105 (May 91; 135 m)	0.056	2.18

cells, we will refer to these peaks as "G_1" and "G_2", corresponding to the analogous stages in the eukaryotic cell cycle (VAULOT and PARTENSKY, 1992a). In contrast, at 117 m (Fig. 5B), the two Chl populations had slightly different DNA contents. Using the Chl fluorescence to discriminate the DNA of each population (Fig. 5B, insert) we found that the bright Chl cells had about 14% more DNA than the dim Chl cells. This difference was highly significant ($P = 0.000$; Fig. 5).

Synechococcus

Synechococcus cyanobacteria occurred at densities typically two orders of magnitude lower than *Prochlorococcus* (Fig. 3C). The vertical structure of *Synechococcus* abundance was more variable than for either *Prochlorococcus* or picoeukaryotes. Two maxima were generally observed: one at the surface and one deeper with larger concentrations (up to 4400 cell ml^{-1}) that often coincided with the *Prochlorococcus* maximum (Fig. 3 and Table 2). In contrast to *Prochlorococcus*, however, *Synechococcus* abundance decreased rapidly to undetectable levels at, or just below, the DCML. Chl and orange phycoerythrin fluorescence per cell were tightly correlated for all samples ($r = 0.97$; $n = 34$), such that we restrict ourselves to discuss only Chl fluorescence in the following. Chl fluorescence increased 25- to 40-fold from the surface down to the Chl maximum (Fig. 3C). It was twice as high in winter/spring than in summer/fall, except in the surface mixed layer, where we observed a 3-fold annual range (Fig. 3D). This pattern was similar to what we observed for *Prochlorococcus*, for which the seasonal variability was less than 2-fold. *Synechococcus* Chl fluorescence was maximum at approximately 125 m, always at or just below the DCML.

Since oceanic populations of *Synechococcus* have been shown to exhibit varying proportions of phycouribilin (PUB) and phycoerythrobilin (PEB) chromophores (CAMPBELL and ITURRIAGA, 1988; OLSON *et al.*, 1988), we examined *Synechococcus* pigment types by measuring the ratios of phycoerythrin fluorescence emission and excitation (WOOD *et al.*, 1985). The analysis of *Synechococcus* clones from the Woods Hole Culture Collection (WATERBURY *et al.*, 1986) showed that a high ratio of green (525 nm) to orange (575 nm) fluorescence is a good indicator of the presence of PUB (Table 3). Moreover both

green:orange (525:575 nm) emission and 488:all lines (457–515 nm) excitation ratios increase with PUB:PEB ratios (Table 3) in accordance with the wide separation in excitation peaks of the two chromophores (492 vs 542 nm, ONG *et al.*, 1984). The use of these indices for natural populations and cultivated isolates from station ALOHA (Table 3) revealed that they all had very high ratios of green:orange emission and 488:all lines excitation. This suggests that station ALOHA populations contain PUB and have high ratios of PUB:PEB. Within any given profile, the ratio of 488:all lines excitation did not display any significant trend, and the green:orange emission ratio decreased from 0.04–0.06 at the surface to 0.02–0.03 at depth.

Picoeukaryotic algae

The small eukaryotic algae were the least abundant group of picophytoplankton. Typically their abundance ranged from 100 to 1000 cell ml^{-1} slightly lower than that of *Synechococcus*, and increased with depth down to a maximum just above the DCML in summer, fall, and winter, but just below the DCML in spring (Fig. 3E and Table 2). They extended deeper in the water column than *Synechococcus* down to 200 m. The vertical structure of Chl fluorescence for picoeukaryotes showed the same trend as for the other two populations, but the increase with depth was much less pronounced, with at most a 7.5-fold range between surface and deep populations (Fig. 3F). Within the surface mixed layer and below the DCML, Chl fluorescence varied only two-fold over the year.

DISCUSSION

Seasonal variability

Although this paper is not intended to be an investigation of seasonal variability *per se* and is limited to four vertical profiles, one collected each season, all indices point to a fairly stable structure of the picophytoplankton community. This lack of variability can be seen in abundance as well as Chl fluorescence per cell for each of the three groups we examined (Table 2 and Fig. 3). For *Prochlorococcus* 200-m integrated counts, there was a mere 37% difference between the minimum and maximum values (Fig. 6). For *Synechococcus* and picoeukaryotes this difference was larger, 106 and 66% respectively. The stability of the community at station ALOHA is due, most likely, to the absence of winter deep vertical mixing (LEWIS *et al.*, 1988), which translates into a permanent DCML (BIENFANG and SZYPER, 1981; WINN *et al.*, 1991) as in the South Sargasso Sea (SIEGEL *et al.*, 1990). In contrast, off Bermuda winter mixing that occurs north of 32°N induces the destruction of the DCML (MENZEL and RYTHER, 1960). In a similar manner, deep convection in the Mediterranean Sea brings nutrients to the surface layer in winter (GASCARD, 1978; VAULOT and PARTENSKY, 1992a) so there is not a permanent DCML throughout the year. The absence of a clear annual pattern off Hawaii does not mean, however, that temporal variability is absent. Internal waves (MCGOWAN and HAYWARD, 1978) and short term events, such as storms (DITULLIO and LAWS, 1991), may have a definite effect on the phytoplankton community in the subtropical Pacific. For our present purpose, the absence of a clear seasonal pattern may allow us to refer in the following to the "typical" structure of the picophytoplankton community at station ALOHA.

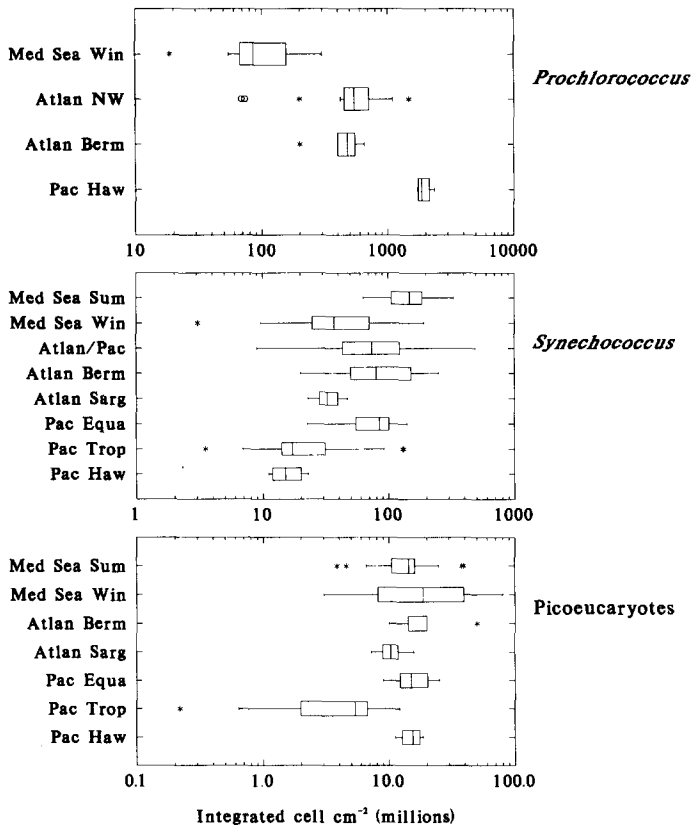

Fig. 6. Global comparison of integrated abundances (0–200 m) expressed in 10^6 cell cm^{-2} for *Prochlorococcus*, *Synechococcus* and picoeukaryotes in the Pacific, Atlantic, and Mediterranean Sea (see Table 4 for data origin). Boxes encompass 50% of the available data for each set with middle lines corresponding to medians. Whiskers enclose the whole data range, with the exception of outliers (represented by stars and circles) that fall more than two box widths away from the median.

Depth-integrated abundances

To compare our observations with those collected previously in the Pacific and Atlantic Oceans, as well as the Mediterranean Sea, we first examined cell abundances integrated over the 200-m water column (Table 4 and Fig. 6). This appeared more meaningful and more robust statistically than comparing the cell concentrations of individual samples. For this purpose, we used data obtained by flow cytometry except for the tropical Pacific for which only epifluorescence counts are available. Integrated *Prochlorococcus* concentrations are clearly higher than average (2×10^9 cell cm^{-2}) and are half an order of magnitude larger than off Bermuda (Olson *et al.*, 1990a). Integrated values nearing those at station ALOHA are an exception in the North Atlantic and have been found only near the Gulf Stream (Olson *et al.*, 1990a; Fig. 6). In contrast, *Synechococcus* integrated abundances at station ALOHA are among the lowest recorded in the three areas examined. They fall, however, in the range observed in the subtropical South Pacific by Blanchot *et al.* (1992); these authors demonstrated that the increase seen at the equator

Table 4. Origin of data plotted in Fig. 6 (integrated counts). n is the number of profiles included

Label	Oceanic region	Latitude range	*n*	Method	Source
Pac Haw	subtropical North Pacific	23°N	4	flow cytometry	This study
Pac Trop*	subtropical South Pacific	20°S–5°N	39	epifluorescence microscopy	BLANCHOT *et al.* (1992)
Pac Eq	equatorial Pacific	6°S–4°N	11	epifluorescence microscopy	BLANCHOT *et al.* (1992)
Atlan Sarg	Sargasso Sea	20°N–30°N	23	flow cytometry	NEVEUX *et al.* (1989)
Atlan Berm	Sargasso Sea	32°N	7	flow cytometry	OLSON *et al.* (1990a)
Atlan NW	North-West Atlantic	27°N–8°N	17	flow cytometry	OLSON *et al.* (1990a)
Atlan/Pac	North Atlantic and East Pacific	7°N–40°N	71	flow cytometry	OLSON *et al.* (1990b)
Med Sea Win†	North-West Mediterranean Sea	41°N–3°N	15	flow cytometry	VAULOT *et al.* (1990)
Med Sea Sum‡	North-West Mediterranean Sea	41°N–3°N	15	flow cytometry	VAULOT and PARTENSKY (1992b)

*Equatorial data included when no upwelling (1987).
†Winter data.
‡Summer data.

(Fig. 6) was linked to upwelling. Figure 6 clearly illustrates that *Prochlorococcus* and *Synechococcus* have overall opposing trends: when one is high the other is low and *vice versa*. The very high *Prochlorococcus*:*Synechococcus* abundance ratio (≈100) observed at station ALOHA is consistent with pigment analyses at the DCML, although the ratio of the *Prochlorococcus* to *Synechococcus* contributions to Chl *a* is much lower (≈2, as determined by the pigment algorithm from LETELIER *et al.*, in press). These opposing trends can be explained by the observations of OLSON *et al.* (1990a,b) who found that integrated *Synechococcus* concentrations decrease as the depth of nitracline increases, while *Prochlorococcus* concentrations track nitracline depth. The high concentration is therefore probably a consequence of the generally deep nitracline at station ALOHA (Fig. 2).

It is noteworthy that, unlike *Prochlorococcus* and *Synechococcus*, picoeukaryotes integrated counts are very comparable to those observed elsewhere, in particular near Bermuda. In fact, picoeukaryotes display amazingly little ocean-wide variability. The only exception appears to be the tropical Pacific where concentrations are much lower (BLANCHOT *et al.*, 1992), perhaps an artifact of the epifluorescence microscopy counting method (Table 4) that is known to induce picoeukaryote cell breakage and Chl degradation (LI and WOOD, 1988).

Vertical structure

The distinctiveness of the central Pacific in terms of integrated abundances for the photosynthetic prokaryotes is also manifested in the vertical structure, in particular for *Prochlorococcus*.

In previous reports, two types of vertical distributions for *Prochlorococcus* have been described. Initially, they were observed to exhibit a bell-shaped profile with a maximum near the DCML and at least an order of magnitude decrease between the concentration in surface and at the maximum (e.g. in the Sargasso Sea in summer, OLSON *et al.*, 1990a). In this case, the depth of the maximum appeared to be related directly to that of the nitracline (OLSON *et al.*, 1990a). A second type of distribution featured uniform abundances of *Prochlorococcus* in the surface mixed layer and decline below, as seen for example in winter in the Sargasso Sea (OLSON *et al.*, 1990a) and in the Mediterranean Sea (VAULOT *et al.*, 1990), and more recently at the equator (CHAVEZ *et al.*, 1991). For this latter profile type, nitrates were in general present in the surface layer. These two types of distributions suggested that *Prochlorococcus* were sensitive to nitrogen depletion but had a strong capacity to photoacclimate at low light levels. This explained their success at tracking the nitracline, even when it reached depths close to the 0.1% light level. This hypothesis was recently strengthened by the observation that *Prochlorococcus* cell cycle was controlled by nitrogen availability in the Mediterranean Sea (VAULOT and PARTENSKY, 1992a).

What we have observed in the subtropical Pacific, however, is a new type of distribution which combines aspects of both previous types: *Prochlorococcus* abundant in the surface mixed layer, and also in the DCML where they exhibit a slight maximum (Fig. 3). The difference between surface and DCML abundances remained small in all cases, less than 2-fold (Fig. 3). Both the greater maximum abundances than in other regions and the absence of a minimum in surface account for the much higher integrated abundances in the subtropical Pacific (Fig. 6).

While the large abundances observed at depth conform to the current paradigm of high nutrient/low light acclimation of *Prochlorococcus*, those observed in the surface mixed layer, where nitrates were always below detection (WINN *et al.*, 1991, 1993), do not fit. A way to solve this paradox is to hypothesize the existence of two populations, one acclimated to low light and similar to those observed in other oceanic regions, one acclimated to low nutrients, not observed elsewhere. This idea is supported by the bimodal distribution of *Prochlorococcus* Chl fluorescence observed near the DCML (Fig. 4) that could reflect a difference in pigment composition: LETELIER *et al.* (in press) found that the divinyl Chl *b*:*a* ratio of DCML *Prochlorococcus* was larger in the 0.65–1.2 μm size range (which corresponds presumably to the larger and brighter cells) than in the 0.22–0.65 μm fraction (smaller, dimmer cells). Bimodality in pigment fluorescence distribution has been observed previously for *Synechococcus* by OLSON *et al.* (1988, 1990b). These authors demonstrated that, in this case, the dim and bright populations were genetically different, since sorted cells of each type maintained their relative fluorescence characteristics once established in culture, and belonged to different pigment types (respectively to the low and high PUB types, OLSON *et al.*, 1988). Such dual *Synechococcus* populations were mostly observed in relatively shallow coastal waters (OLSON *et al.*, 1990b). In the case of *Prochlorococcus*, all populations observed to date have been unimodal (e.g. CHISHOLM *et al.*, 1988; OLSON *et al.*, 1990a; VAULOT *et al.*, 1990), with the exception of OLSON *et al.*'s (1991) report of a case of bimodality within the nitracline in the Sargasso Sea.

The evidence which indicates genetic differences do exist between the two *Prochlorococcus* populations in the Pacific is the 14% discrepancy we were able to detect in cell DNA fluorescence (very likely reflecting a real difference in cell DNA content since one population served as an internal standard for the other). It must not be concluded hastily, however, that the dim (or bright) population is genetically identical across all depths.

There could well be several dim (or bright) populations. Only high resolution vertical profiles and proper immunological or genetic probes (CAMPBELL and CARPENTER, 1987; DE LONG, 1991) could resolve this question. If the dim and bright populations are indeed genetically distinct, they probably have optimum growth rates at different depths. If we take the example of HOT-25 (Fig. 4), the dim population reached a maximum abundance at 77 m and the bright one at 102 m (not shown, note that Fig. 4 shows cell density, not absolute abundances). In a situation where vertical diffusion dominates over vertical and horizontal advections, these depths must correspond to depths where cell populations display the highest growth rate (division minus grazing minus death). At 77 and 102 m NO_3^- concentrations were calculated to be 0.14 and 0.53 μM, respectively, and light levels estimated to be 1.0 and 0.5% of I_0, respectively, which illustrates the potential differences in the optimum growth conditions of the dim and bright populations, the former being adapted to lower nutrients/higher light and the latter to higher nutrients/lower light. Such differences in the growth rates of genetically different strains of *Prochlorococcus* have been demonstrated recently by PARTENSKY *et al.* (1993). The depth range where such bimodality may be observed must be a complex function of the position of the nitracline, the light profile as well as mixing processes linked in particular to internal waves (MCGOWAN and HAYWARD, 1978). It is noteworthy that we have not observed bimodality for *Synechococcus*, confirming that the ecology of these two prokaryotes is fairly distinct at station ALOHA (see below).

In contrast to cell abundances, the trend in the vertical distribution of *Prochlorococcus* Chl fluorescence is very similar to previous reports, if one omits the bimodality discussed above. In particular, the 50-fold increase between the dimmest and the brightest cells falls into the range observed by OLSON *et al.* (1990a). A novel observation is the several-fold increase in the 488:457 excitation ratio for Chl fluorescence, which is indicative of an increase in the Chl *b*:*a* ratio with depth (CAMPBELL *et al.*, 1992; BIDIGARE *et al.*, in preparation) and is a feature in agreement with culture studies (PARTENSKY *et al.*, 1993).

Unlike *Prochlorococcus*, the vertical structure of *Synechococcus* fits within the framework of previously reported distributions in oligotrophic regions, in particular in the Atlantic Ocean (OLSON *et al.*, 1990b) and the subtropical Pacific (BLANCHOT *et al.*, 1992). Features such as the slight maximum located just above the DCML as well as above the *Prochlorococcus* maximum, the rapid disappearance of cells below the base of the DCML, the large increase in pigment fluorescence already have been described.

Previously, it was reported that *Synechococcus* strains with high PUB:PEB ratios dominate in the Atlantic Ocean (CAMPBELL and ITURRIAGA, 1988; OLSON *et al.*, 1988) as well as in the northwestern Mediterranean Sea (LANTOINE, 1991). This is also true in the central subtropical Pacific (Table 3). The absence of any trend with depth for the 488:all lines excitation ratio (which we assume covaries with the PUB:PEB ratio) is similar to OLSON *et al.*'s (1990b) observations in the Atlantic. In contrast, LANTOINE (1991) using spectrofluorometry observed a slight increase in the PUB:PEB ratio with depth in the Mediterranean Sea: this conforms to the expectation of an increase in blue absorption with depth. The decrease with depth we observed in the green:orange emission ratio is more difficult to interpret.

Like *Synechococcus*, picoeukaryotes at station ALOHA exhibit vertical distributions of cell abundances and red fluorescence similar to that observed elsewhere in oligotrophic areas, characterized in particular by a cell maximum very close to the DCML (GLOVER *et al.*, 1986; LI and WOOD, 1988; EPPLEY *et al.*, 1988; BLANCHOT *et al.*, 1992). We have no data

to assess their exact taxonomic affiliation; however, pigment analyses at station ALOHA (LETELIER *et al.*, in press) suggest that a majority could belong to the Prymnesiophyceae or the Chrysophyceae.

CONCLUSION

The photosynthetic picoplankton community structure in the subtropical central Pacific Ocean north of Hawaii is unlike previously described communities and is distinguished by the following main features: absence of large seasonal variability, numerical dominance of *Prochlorococcus* over *Synechococcus*, presence of *Prochlorococcus* both in the surface mixed layer and at the DCML, existence of two *Prochlorococcus* populations, similarity of picoeukaryote abundances with other oceanic regions. Are these data only valid for station ALOHA or are they applicable to the whole of the subtropical Pacific? HERBLAND and VOITURIEZ (1979) demonstrated more than a decade ago that in the tropical Atlantic, biological, chemical, and physical features of the euphotic layer were statistically related, which allowed them to define what they called a typical tropical structure. Later, HERBLAND *et al.* (1985) went on to show that the size distribution of the phytoplankton was very stable for such typical tropical structures throughout the whole tropical Atlantic. More recently, LE BOUTEILLER *et al.* (1992) established that the same was true in the Pacific and that no real differences could be detected between the two oceans. Pigment analyses across the North Pacific central gyre (ONDRUSEK *et al.*, 1991) confirm that stability of the phytoplankton community structure. LE BOUTEILLER *et al.* (1992) found that cells smaller than 1 μm made up systematically more than 50% of the Chl *a* when NO_3^- was below 0.1 μM and less than 50% when the latter condition was not met (i.e. in general below the DCML or in the equatorial upwelling). Although these authors did not estimate *Prochlorococcus* abundance, they identified the <1 μm fraction with photosynthetic prokaryotes and the >1 μm with picoeukaryotes (microalgae). Their data are consistent with the data obtained at station ALOHA that point to a dominance of *Prochlorococcus* above the DCML and of picoeukaryotes below (CAMPBELL *et al.*, unpublished) and suggest that the structure we observed is probably typical of the whole subtropical Pacific, wherever no deep mixing takes place.

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