Hydrobiologia **352:** 219–230, 1997.
Y.-S. Wong & N. F.-Y. Tam (eds), Asia–Pacific Conference on Science and Management of Coastal Environment.

Spatial variations of size-fractionated Chlorophyll, Cyanobacteria and Heterotrophic bacteria in the Central and Western Pacific

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Key words: Size-fraction, Chlorophyll, Cyanobacteria, Heterotrophic bacteria, Pacific

Abstract

Geographic and vertical variations of size-fractionated (0.2–1 μm, 1–10 μm, and >10 μm) Chlorophyll *a* (Chl.a) concentration, cyanobacteria abundance and heterotrophic bacteria abundance were investigated at 13 stations from 4°S, 160°W to 30°N, 140°E in November 1993. The results indicated a geographic distribution pattern of these parameters with instances of high values occurring in the equatorial region and offshore areas, and with instance of low values occurring in the oligotrophic regions where nutrients were almost undetectable. Cyanobacteria showed the highest geographic variation (ranging from 27×10^3 to $16{,}582\times10^3$ cell l^{-1}), followed by Chl.a (ranging from 0.048 to 0.178 μg l^{-1}), and heterotrophic bacteria (ranging from 2.84×10^3 to 6.50×10^5 cell l^{-1}). Positive correlations were observed between nutrients and Chl.a abundance. Correspondences of cyanobacteria and heterotrophic bacteria abundances to nutrients were less significant than that of Chl.a. The total Chl.a was accounted for 1.0–30.9%, 35.9–53.7%, and 28.1–57.3% by the >10 μm, 1–10 μm and 0.2–1 μm fractions respectively. Correlation between size-fractionated Chl.a and nutrients suggest that the larger the cell size, the more nutrient-dependent growth and production of the organism. The ratio of pheophytin to chlorophyll implys that more than half of the >10 μm and about one third of the 1–10 μm pigment-containing particles in the oligotrophic region were non-living fragments, while most of the 1–10 μm fraction was living cells. In the depth profiles, cyanobacteria were distributed mainly in the surface layer, whereas heterotrophic bacteria were abundant from surface to below the euphotic zone. Chl.a peaked at the surface layer (0–20 m) in the equatorial area and at the nitracline (75–100 m) in the oligotrophic regions. Cyanobacteria were not the principle component of the picoplankton. The carbon biomass ratio of heterotroph to phytoplankton was greater than 1 in the eutrophic area and lower than 1 in oligotrophic waters.

Introduction

Phytoplankton, cyanobacteria and heterotrophic bacteria are the principal components of the basic communities in oceanic environments: their dynamics are of crucial importance to the understanding of the structures and functions of marine ecosystems, especially with respect to material cycling and climate changes (Cho & Azam, 1990; Krupatkina, 1990; SCOR, 1990). The Pacific ocean is the largest water body in the world; it can be divided into two divisions according to the nutrient availability: the eutrophic region and the oligotrophic region. The central equatorial area is a typical upwelling-driven eutrophic area, and coastal areas constitute another kind of eutrophic area due to the input of nutrients from the land. However, the majority of the open ocean is made up of oligotrophic waters. It is, therefore, of interest to investigate the spatial dynamics of the aforementioned microorganisms from the central Pacific to coastal areas and to determine the controlling factors for their standing biomass.

There has been a large number of investigations on the geographic distribution and temporary variation of phytoplankton around the world's oceans, but most of them only dealt with whole phytoplankton assemblages. Size fractionated biomass measurements

are still rare especially in the Pacific Ocean (Chavez, 1989; Bouteiller et al., 1992). Furthermore, glass fiber filters (Whatman GF/F), rather than Nuclepore filters, were often used, allowing some picoplankton species to escape collection and thus leading to an underestimation of Chlorophyll *a* (hereafter, Chl.a) concentration in subtropical and transition waters by a factor of two to four (Dickson & Wheeler, 1993). Also, autotrophic and heterotrophic organisms are often studied separately. In addition, most of the previous studies were conducted over relatively small areas. Basin-wide investigations however are limited because either they involve time differences which limit data comparability or because they require a heavy outlay. The present study is therefore aimed at (1) displaying the geographic variation based on the synchronous measurement of size-fractionated chlorophyll *a* concentration, cyanobacteria and heterotrophic bacteria abundance on a transregional scale from the Central Pacific to the Northwest Pacific; and (2) outlining the vertical profiles of these three indices for microorganisms in typical eutrophic and oligotrophic areas, so as to understand their general distribution pattern and the controlling mechanism for that distribution pattern.

Description of study sites and sampling techniques

This study was conducted during a cruise of the Hakuho Maru of Tokyo University in November 1993. There were 13 sampling sites (Figure 1, with Coordinates in Table 1) from transect lines distributed from the equatorial central Pacific (4° S, 160° W) to the Northwest Pacific (30° N, 42° E). Samples for our spatial dynamic study were collected approximately 10 am daily from the surface water (about 5 m) of Stations 1–12 with an on-board sampling bump. Samples for vertical distribution study were collected from the central equatorial upwelling area (Station 2) at 0, 20, 50, 75, 100 and 150 m, as well as the oligotrophic area (Station 0) at 0, 20, 25, 50, 75, 100, 150, 200 and 300 m with 10-liter Niskin bottles attached to a CTD rosette system (General Oceanics). Sampling depth were determined according to CTD fluorescence profiles monitored by SeaTech Fluorometer.

Materials and methods

Size fractionation of microorganisms

Natural phytoplankton assemblages were divided into 3 size categories: picoplankton (hereafter, Pico; 0.2–1 μm), nanoplankton (Nano; 1–10 μm) and netplankton (Net; >10 μm) respectively. In order to reduce the systematic error from repeated filtering with Nuclepore filters of different pore sizes, size fractionation was performed by filtering respective 1000 ml, 500 ml, and 100 or 200 ml water sample directly onto corresponding 10 μm Nuclepore filter at gravity, and 1 μm and 0.2 μm Nuclepore filters in a vacuum of less than 0.03 MPa respectively. The Nano fraction would, therefore, be obtained by subtracting the >10 μm fraction (Net) from the >1 μm fraction (Net + Nano) and the Pico fraction by subtracting the >1 μm fraction from the >0.2 μm fraction (Net + Nano + Pico). Duplicate measurements were done for each sample. Usually the coefficients of variation are less than 10%. Size fractionation of microorganisms was performed at St. 2 through St. 12.

Determination of pigments

Chl.a and pheophytin (Phe.) in the filter samples were extracted by N, N-Dimenthylformamide which has been shown to be more efficient than acetone (Suzuki & Ishimaru, 1990), and then measured on board by fluorometric techniques using a Turner Design model-10AU fluorometer which was calibrated with commercial Chl.a (Sigma).

Cell counting

Aliquots of 30–50 ml water were subsampled into polycarbonate tubes, which were then fixed with formalin at a final concentration of 2% and stored at 4 °C in a refrigerator, for subsequent numerical counting of cyanobacteria and heterotrophic bacteria. Microscopic examination of these samples was performed within 4 weeks of the fixing at which time the stored samples were filtered onto 0.2 μm black Nuclepore at a vacuum of less than 0.03 MPa. Cyanobacteria were counted by epifluoreoscence method (Wood et al., 1985) with a Zeiss epifluoreoscence microscope set equipped with BP450-490, FT510, and LP515 light filters. Heterotrophic bacteria were stained with 4,6-diamidinno-2-phenylindole (DAPI) (Porter & Feig, 1980) and counted by epifluorescence microscope with

Table 1. Location of 13 stations with contributions of size-fractionated Chlorophyll a to total Chlorophyll a.

Station No.	Longitude	Latitude	Total Chl.a ($\mu g\, l^{-1}$)	Net Chl.a (%)	Nano Chl.a (%)	Pico Chl.a (%)
0	160° 00′ W	5° 00′ N	0.098	–	–	–
1	160° 00′ W	4° 00′ S	0.154	–	–	–
2	159° 00′ W	0°	0.178	30.89	41.01	28.10
3	165° 00′ W	3° 00′ N	0.103	6.80	35.92	57.28
4	171° 00′ W	6° 00′ N	0.074	10.81	39.19	50.00
5	176° 30′ W	8° 58′ N	0.048	14.58	45.83	39.58
6	177° 14′ E	12° 08′ N	0.069	8.69	53.62	37.68
7	171° 12′ E	15° 08′ N	0.079	6.33	48.10	45.57
8	165° 00′ E	18° 16′ N	0.077	7.79	46.75	45.45
9	159° 00′ E	21° 07′ N	0.079	8.86	48.10	43.04
10	153° 43′ E	24° 23′ N	0.067	8.96	44.78	46.29
11	147° 06′ E	27° 00′ N	0.105	0.90	50.94	48.11
12	141° 36′ E	29° 18′ N	0.127	11.03	45.67	43.30
mean			0.102	10.51	45.45	44.04

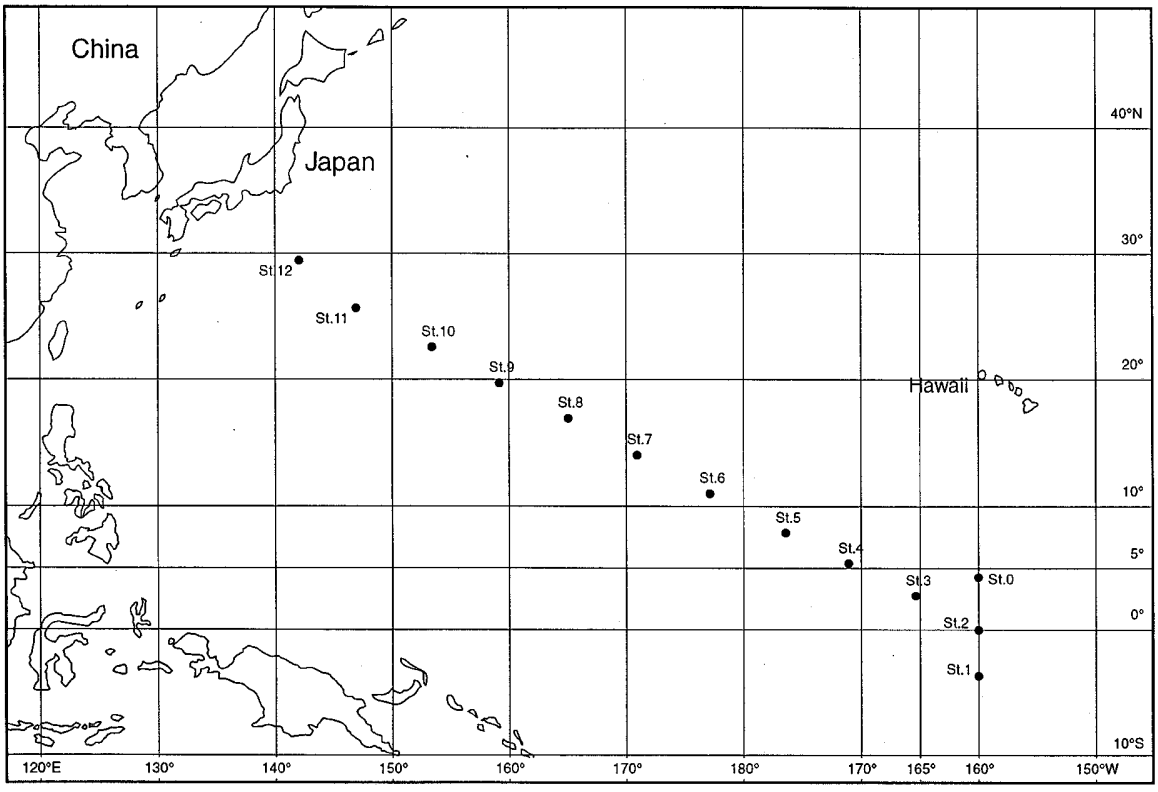

Figure 1. Locations of sampling sites (see Table 1 for coordinates). Stations 0 and 2 were designed especially for vertical variation study.

a FT390, and LP395 filter set. Heterotrophic bacteria were divided into 'long-shaped' and 'round shaped' groups according to whether the ratio of long axis of the cell to short axis of the cell was obviously greater than 2.

Determination of nutrients

Nitrate, nitrite, ammonia and phosphate concentrations were measured with a Technicon II autoanalyzer according to the method described by Parsons et al. (1984). Nutrient detection limits were 0.08, 0.01, 0.01 and 0.02 μmol l^{-1} for nitrate, nitrite, ammonia and phosphate respectively.

Results

1. Geographic variations of nutrients

The geographic distribution of temperature and salinity are displayed in Figure 2. The sea surface temperature was relatively low and the salinity relatively high in the equatorial area which indicated the existence of upwelling. The geographic variations of such nutrients as nitrate, nitrite, ammonia, and phosphate are shown in Figure 3. The univariate statistics (mean, standard deviation and range) for each variable among 12 stations are summarized in Table 2. Along the cruise from St. 1 to St. 12. nitrate varied from undetectable to 2.30 μmol l^{-1} with two peaks occurring at St. 2 and around St. 12. St. 2 is within the equatorial upwelling area where new nitrogen, especially nitrate, (Dugdale & Goering, 1967) is upwelled to the surface layer from below the thermocline (about 100 m, refer to Figure 7a). Here nitrate had the highest concentration among all the investigation sites. The secondary peak at St. 12. was apparently due to the land nutrient input since there was an inshore-direction increasing trend from St. 10 to St. 12. The concentrations of nitrite and phosphate followed the same pattern as for nitrate, ranging from undetectable to 0.59 μmol l^{-1} and from undetectable to 0.47 μmol l^{-1} respectively. Ammonia, a typical regenerated nitrogen (Dugdale and Goering, 1967), only ranged from undetectable to 0.06 μmol l^{-1}; its geographic coefficients of variation were less significant than that of the other three nutrients investigated.

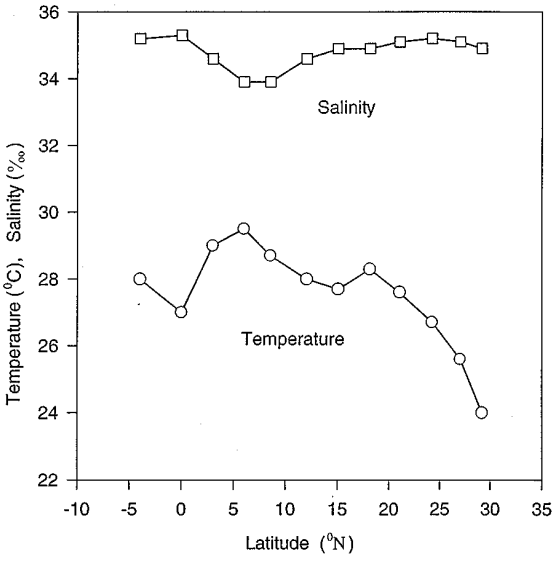

Figure 2. Geographic variations of surface temperature and salinity. Refer to Figure 1 and Table 1 for the corresponding latitude for each station.

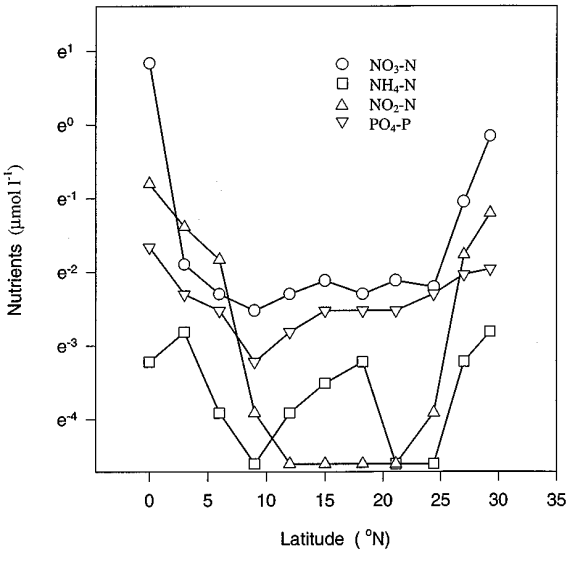

Figure 3. Geographic variations of surface nitrate, nitrite, ammonia and phosphate.

2. Geographic variations of pigments

The geographic variations of total and size-fractionated Chl.a are shown in Figure 4a. For total Chl.a, the peak values occurred at St. 1, 2 and 12, the former two stations are in the equatorial area, and the latter is in an offshore area. The lowest value occurred at St. 5 where the levels of all the nutrients were lowest. Mea-

Table 2. Univariate statistics of temperature, salinity, nitrate, ammonia, phosphate, total and fractionated (Net, Nano, Pico) Chlorophyll a, cyanobacteria, total bacteria and long-shaped heterotrophic bacteria for stations 1 to 12.

	Temp °C	Salinity ‰	NO_3 μ mol l^{-1}	NH_4 μ mol l^{-1}	PO_4 μ mol l^{-1}	Total Chl.a $\mu g\, l^{-1}$	Net Chl.a $\mu g\, l^{-1}$	Nano Chl.a $\mu g\, l^{-1}$	Pico Chl.a $\mu g\, l^{-1}$	Cya Bact $\times 10^7$ cell l^{-1}	Total Bact $\times 10^8$ cell l^{-1}	Long Bact $\times 10^8$ cell l^{-1}
Mean	27.51	34.80	0.38	0.03	0.14	0.10	0.01	0.04	0.04	0.30	4.01	0.37
SD	1.53	0.47	0.64	0.02	0.16	0.04	0.01	0.01	0.01	0.47	1.18	0.12
Min	24.00	33.90	UD#	UD#	UD#	0.05	0.00	0.02	0.02	0.00	2.84	0.11
Max	29.50	35.30	2.30	0.06	0.47	0.18	0.06	0.07	0.07	1.66	6.50	0.52
CV∗	0.06	0.01	1.68	0.67	1.14	0.40	1.34	0.36	0.35	1.61	0.29	0.33

∗ CV: Coefficient of variation
UD: nutrients undetectable

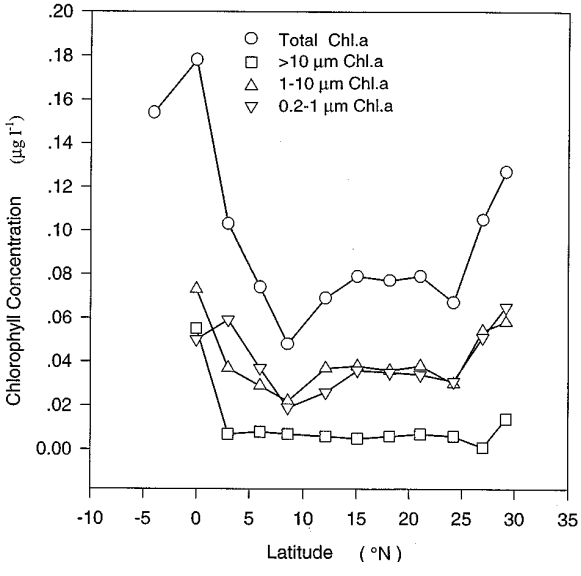

Figure 4a

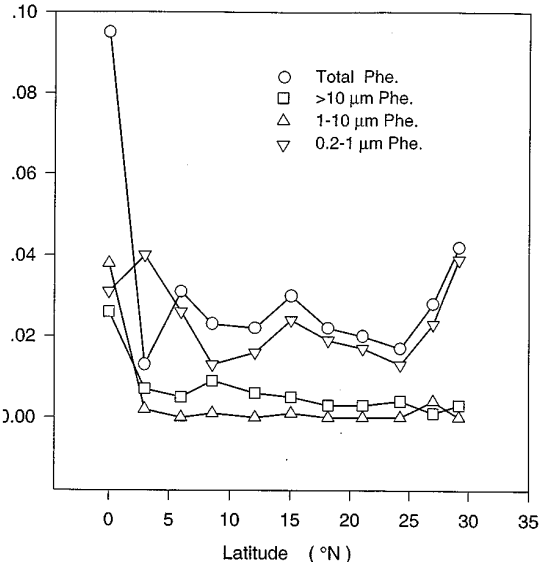

Figure 4b

Figure 4. Geographic variations of surface chlorophyll *a* (4a) and pheophytin (4b).

surements of the three Chl.a size-fractions followed the same geographic distribution trend as the total, but with different variabilities. The coefficients of variation for the Net, Nano and Pico fractions were 1.34, 0.36 and 0.35 respectively (Table 2). Regarding contributions of different fractions to the total Chl.a, the Net fraction was on average responsible for only 10.53% of the total, the remaining contribution (approximately 90%) was shared by Nano and Pico fractions (Table 1). The Net fraction also had the most geographic variability in terms of its relative contribution (%), which peaked where the nutrients were relatively abundant. Phe. (Figure 4b) had a geographic variation pattern similar to that of Chl.a, but the variations in different size fractions were quite different. The ratio of Phe. to Chl.a (Table 2) indicates that almost half of the Net fraction and about 1/3 of the Pico fraction were non-living organic particles, whereas most of the particulate matter in the Nano fraction was living organisms.

Significant correlations between Chl.a and nutrients were observed (Figures 5a, 5b and 5c). Nitrate was significantly correlated to the larger fractions (Net and Nano), but not to the smaller fraction (Pico) (Figure 5a). On the other hand, ammonia correlated better with the smaller fractions (Pico and Nano) than with the larger fraction (Net) (Figure 5b). Among all the nutrients, phosphate correlated significantly with total Chl.a as well as each of the three fractions (Figure 5c).

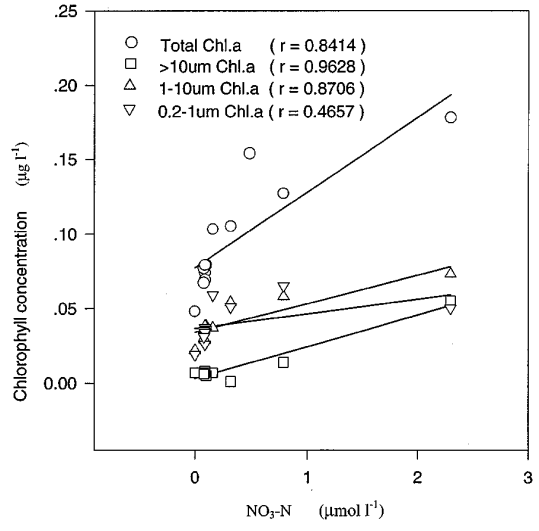

Figure 5a.

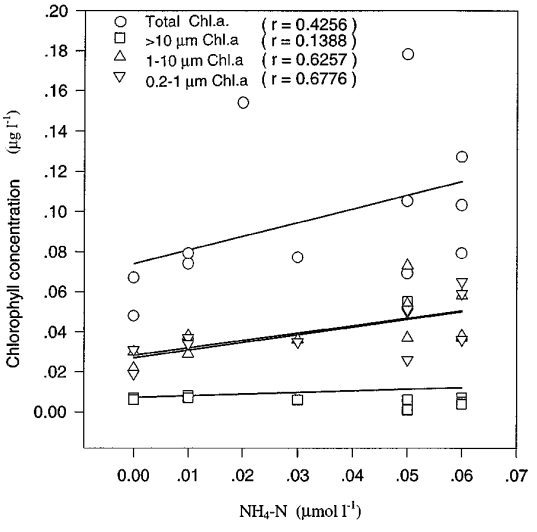

Figure 5b.

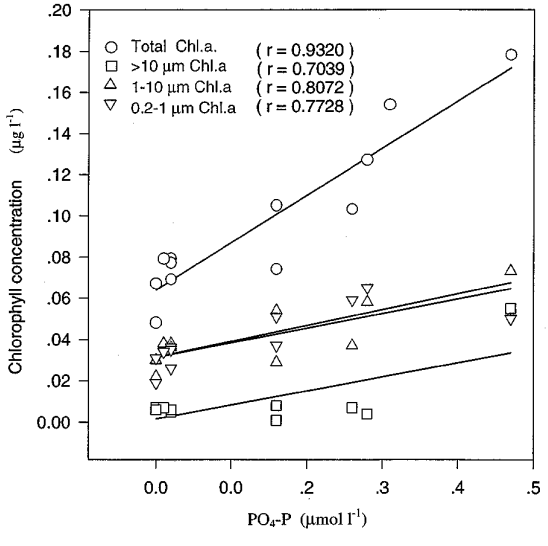

Figure 5c.

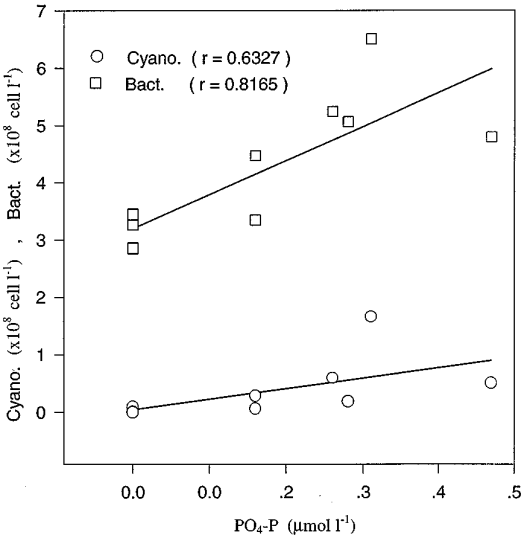

Figure 5d.

Figure 5. Correlations between chlorophyll *a* and nitrate (5a), chlorophyll *a* and ammonia (5b), chlorophyll *a* and phosphate (5c), and Correlation between cyanobacteria, heterotrophic bacteria and phosphate (5d).

3. Geographic distribution of cyanobacteria and heterotrophic bacteria

The abundance of cyanobacteria varied from the order of magnitude of 10^5 cell l^{-1} in the vast oligotrophic region (St. 5 through St. 11) to the order of magnitude of 10^7 cell l^{-1} in the equatorial area (Figure 6a). The coefficient of variation for geographic distribution was as high as 1.61 (Table 2) which was the highest among all the biological parameters investigated. The abundance of heterotrophic bacteria, however, was less geographically variable ranging from 2.85×10^8 to 6.50×10^8 cell l^{-1} with a mean of 4.0×10^8 cell l^{-1} and a coefficient of variation of 0.29 (Figure 6b).

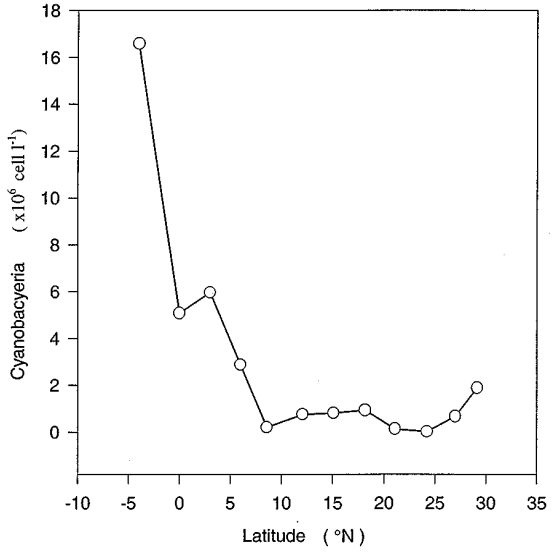

Figure 6a.

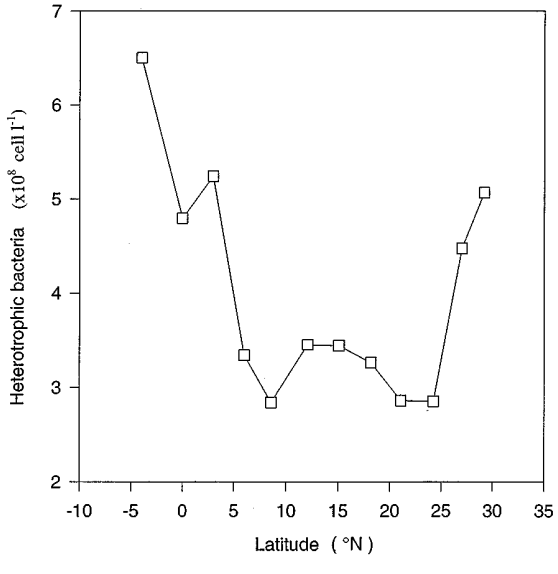

Figure 6b.

Among the heterotrophic bacteria, most of them were round-shaped while about 10% of them were long-shaped (possibly bacillus and vibrio). The percentages of the long-shaped bacteria were higher in the oligotrophic area than in the equatorial region (Figure 6c). Correlation analyses between cyanobacteria, heterotrophic bacteria and nutrients indicated that only phosphate correlated significantly with these microorganisms (Figure 5d).

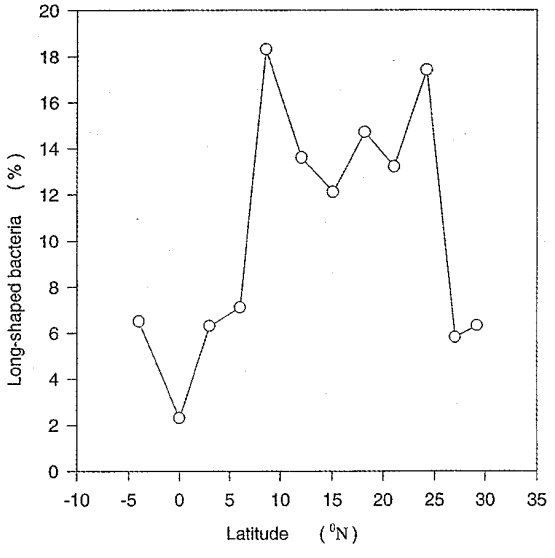

Figure 6c.

Figure 6. Geographic variations of cyanobacteria (6a), heterotrophic bacteria (6b) and the relative abundance of the long-shaped bacteria (6c).

4. Vertical distribution of nutrients

St. 0 is a typical oligotrophic site and St. 2 is a typical equatorial upwelling eutrophic site. The sea surface temperature at St. 2 was slightly lower than at St. 0, but the thermoclines at both stations were almost identical (Figure 7a). The vertical distribution of nitrate, and to a lesser extent, phosphate were characterized by a sharp variation at the thermocline (Figure 7b). In the oligotrophic region, nutrients commonly found in the euphotic zone were very limited. Phosphate was about 0.16 μmol l^{-1}, and nitrate and ammonia were essentially undetectable. Below the thermocline, nitrate increased rapidly and reached a level of 30 to 35 μmol l^{-1}, at a depth of 200 to 300 m. Phosphate also increased slightly with increasing depth, but ammonia was uniform throughout the water column. The vertical distribution of nutrients in the equatorial area was quite different from that in the oligotrophic region. First, all the nutrients were relatively abundant in the euphotic zone, the average concentrations of nitrate, ammonia and phosphate being 4.2, 0.04 and 0.52 μmol l^{-1} respectively. Second, the depth profiles for nitrate and phosphate were less drastic since the upwelling brought 'new nutrients' to the upper layer which reduced the differences between the layers above and below the thermocline. On the other hand,

ammonia was undetectable below the euphotic zone, this demonstrated the typical depth profile for regenerated nitrogen.

5. Vertical distribution of chlorophyll a

Figure 7c shows the vertical distribution of Chl.a in both the oligotrophic region and the equatorial region. With regard to the former case, the concentration of Chl.a in the upper layer (<50 m) was very low, and it decreased slowly with increasing depth from 0.091 μg l^{-1} at the surface to 0.074 μg l^{-1} at a depth of 50 m, then increased abruptly to 0.32 μg l^{-1} over the thermocline, after which it again decreased gradually to undetectable at 200 m. In the equatorial region, the vertical distribution pattern of Chl.a was much more simple. Chl.a peaked at the surface layer (<50 m) with a high value of 0.043 μg l^{-1}, and then gradually decreased to less than 0.02 μg l^{-1} at a depth of 150 m.

6. Vertical distribution of cyanobacteria and heterotrophic bacteria

The depth profile of cyanobacteria was similar to that of Chl.a, however, cyanobacteria distributed shallower than did Chl.a at both oligotrophic sites and equatorial sites (Figure 7d). The maximum abundance of cyanobacteria was about 3×10^6 cell l^{-1} in both the oligotrophic region and the equatorial area, but at different depths. The vertical distribution pattern of heterotrophic bacteria was similar to those of Chl.a in both the equatorial region and the oligotrophic area, but heterotrophic bacteria distributed much deeper than that of Chl.a. Below 200 m, Chl.a was undectable, and heterotrophic bacteria was still abundant. The maximum abundance of heterotrophic bacteria was about 20×10^6 cell l^{-1} both in the oligotrophic region and in the equatorial area.

Discussion

Factors controlling phytoplankton abundance

For the geographic distribution, correlation analysis (Figures 5a, 5b and 5c) indicated that phytoplankton abundance was basically controlled by nutrient availability. For the vertical distribution, in the oligotrophic area, the low phytoplankton biomass (as shown by Chl.a concentration) in the upper layer was apparently due to a lack of nutrients. Given the same nutrient conditions, the decrease of Chl.a in this layer was believed to be due to a decrease in the availability of light. The sudden increase of Chl.a at the thermocline layer, though partially due to higher Chl.a content per cell (light- adaption), was no doubt resulted principally from the abundant supply of nutrients. The subsequent decrease was again caused by the limitation of the availability of light. On the other hand, in the equatorial upwelling area, with sufficient nutrient supply, the phytoplankton depth profile was basically controlled by light availability. In comparison with other normal eutrophic waters, Chl.a in equatorial Pacific waters was still low due to lack of soluble iron as suggested by Martin et al. (1994).

Table 3. The ratio of Pheophytin to Chlorophyll a.

Station No.	Total Chl.a (%)	Net Chl.a (%)	Nano Chl.a (%)	Pico Chl.a (%)
2	53.4	47.3	52.1	62.0
3	12.6	100.0	5.4	67.8
4	41.9	62.5	0.0	70.3
5	47.9	128.6	4.5	68.4
6	31.9	100.0	0.0	61.5
7	38.0	100.0	2.6	66.7
8	28.6	50.0	UD#	54.3
9	25.3	42.9	UD#	50.0
10	25.4	66.7	UD#	41.9
11	26.7	100.0	7.4	45.1
12	33.1	75.0	0.0	60.0
Mean	33.16	79.36	9.00	58.91

UD — Pheophytin was undetectable.

The size structure of phytoplankton

Since the size structure of microorganisms as well as species composition is of crucial importance to the energy flow pathway, it has become one of the focuses of recent studies in marine productivity. Previous research has revealed that picoplankton (usually 0.2–2 μm) accounts for about 40–80% of the total phytoplankton in terms of both biomass and productivity in oligotrophic regions (Grandinger et al., 1993; Gomes et al., 1993; Odate & Fukuchi, 1994) and about 30–50% in coastal waters (Jiao & Wang, 1994; Tata et al., 1994). In the present study, the Pico fraction (<1 μm) accounted for 45% of the total Chl.a, and the Net fraction accounted for only 10% of that total. This supports the results of a comparative study which found that surface Chl.a in the North Pacific measured with 0.2 μm

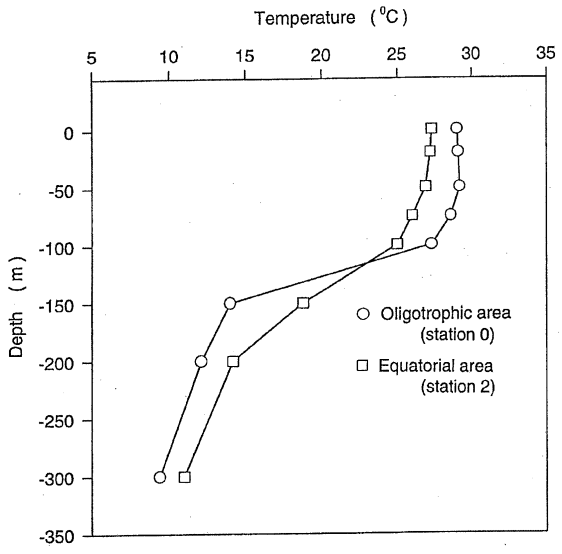

Figure 7a.

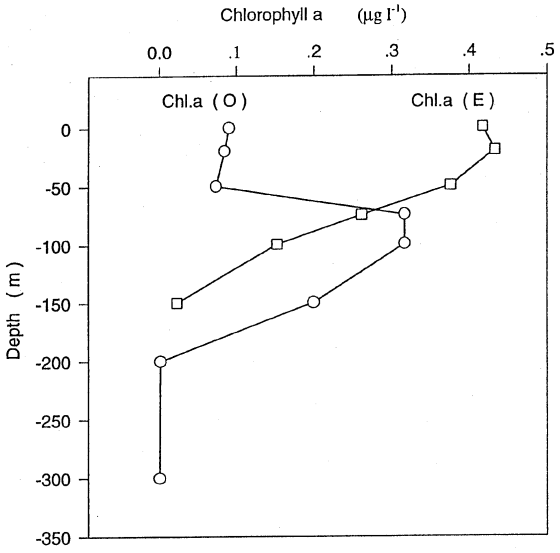

Figure 7c.

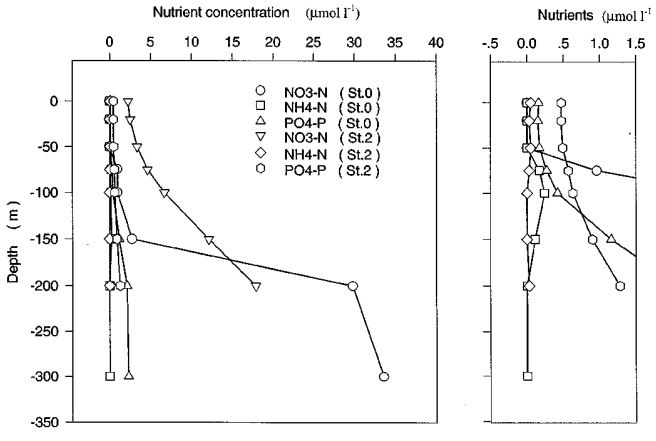

Figure 7b.

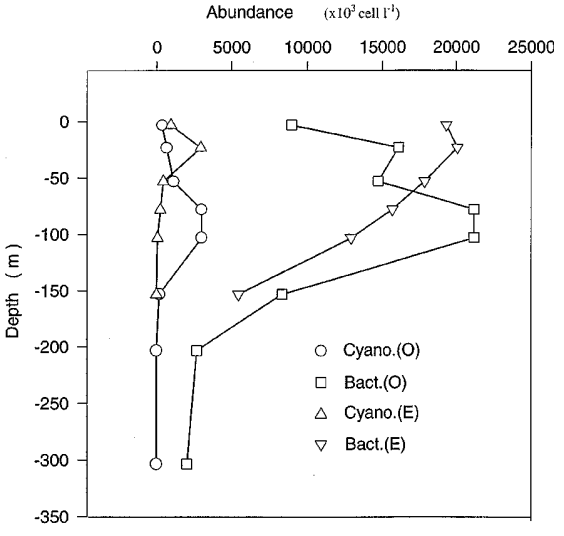

Figure 7d.

Figure 7. Vertical distribution of temperature (7a), nutrients (7b), Chlorophyll *a* (7c), cyanobacteria and heterotrophic bacteria (7d) in equatorial (St. 2) and oligotrophic (St. 0) areas.

Nuclepore was up to four times higher than that measured with Whatman GF/F filters which are extensively used for measurement of Chl.a (Dickson & Wheeler, 1993). With respect to the common concept for size fractionation, picoplankton (0.2–2 μm) would be even more dominant and netplankton (20–200 μm) might be less significant in the pelagic ocean since our size fractionation was based upon 1 μm and 10 μm Nuclepore filters. In fact, an inverse relationship between percentage of picoplankton and total Chl.a was observed, and netplankton showed a decreasing trend with increasing oligotrophic conditions. This confirmed the idea that larger phytoplankton species are associated with eutrophic waters, whereas smaller microorganisms are

dominant in oligotrophic waters (Chisholm, 1992). Moreover, the correspondence of fractionated Chl.a to nitrate and to ammonia were in inverse order: the significance of the correlation coefficient for nitrate formed a Net>Nano>Pico sequence, but for ammonia it formed a Pico>Nano>Net sequence. This suggests that larger phytoplankton cells prefer new nitrogen to regenerated nitrogen whereas smaller cells prefer regenerated nitrogen to new nitrogen. Moreover, nitrate is relatively abundant in the equatorial region and ammonia is relatively dominant in the oligotrophic region. Therefore, it can be concluded that new production would be based mainly on the larger fractions and regenerated production mainly on the smaller fractions, and that the f-ratio (new production/primary production) would be much higher in eutrophic areas than in oligotrophic areas.

The significance of cyanobacteria

During the past decade, cyanobacteria have been the subject of many studies, and they were regarded as forming the dominant component of picoplankton (Krupatkina, 1990). In some instances, cyanobacterial abundance was actually quite high (e.g., Northern Indian Ocean, Veldhuis & Kraay, 1993). But in the Northern Pacific Ocean, the present results for surface distribution and vertical profile do not support the point that cyanobacteria is the dominant component of the picoplankton. The abundance of cyanobacteria was 1–3 orders of magnitude lower than that of heterotrophic bacteria, and probably the 1 or 2 order of magnitude lower than that of *prochlorococcus*, a newly discovered unicellular procaryote with cell size of about 0.6 μm (Chisholm et al. 1992) which we were not able to examine its abundance but included its biomass in terms of Chl.a into pico fraction. Also, biomass of cyanobacteria would account for very small part of autotrophs in terms of carbon which can be calculated by using the conversion factor of 250 fgC per cell for cyanobacteria (Kana & Glibert, 1987), and 71 for phyto-C/Chl.a. (Campbell & Nolla, 1994) (Table 4). This result coincides with the conclusion of a flowcytometric investigation at the ALOHA station (22°45′N, 158°W) (Campbell & Nolla, 1994). Furthermore, the variability of cyanobacteria abundance is the largest among all the three parameters through Station 1–12 (Table 2). From the geographic distribution and the vertical profile, it can be speculated that the abundance of cyanobacteria was limited by either the availability of light or the availability of nutrients.

The role of heterotrophs and autotrophs

Whether bacteria, phytoplankton, or detritus dominate the food for grazers in the euphotic zone is likely to influence the food web structure, nutrient cycling and sinking flux (Cho & Azam, 1990). In the pelagic ocean, detritus is relatively insignificant. Bird & Kalff (1984) reported a positive correlation between bacterial abundance and Chl.a and demonstrated that from eutrophic to mesotrophic waters, the level of bacteria decreased less slowly than that of Chl.a. In this study, we also found a significant positive correlation between bacteria and Chl.a both for geographic distribution and vertical distribution within the euphotic zone. This suggests a dependence of heterotrophic bacteria on phytoplankton for dissolved organic carbon (DOC) supply and a dependence of phytoplankton on heterotrophic bacteria for nutrient supply. In view of their variability, heterotrophic bacteria are more independent of environmental factors than autotrophic organisms. The biomass ratio of heterotrophic bacteria to phytoplankton in terms of carbon (Table 4) is less than 1 in the equatorial eutrophic area and greater than 1 in the oligotrophic areas. This supports the idea that food webs in eutrophic waters are dominated by the primary producer biomass, while, food webs in oligotrophic waters are dominated by the decomposers (Dortch & Packard, 1989). Cho & Azam (1990) reported that the bacteria biomass in oligotrophic waters is commonly 2–3 times greater than the phytoplankton biomass, and it is a key link in food web structure and nutrient cycling pathways. In comparison, the present data for surface waters show less predominance of heterotrophic bacteria over phytoplankton (Table 4).

Campbell & Nolla (1994) pointed out that it is impossible to discriminate *Prochlorococcus* from heterotrophic bacteria when samples are labeled with DAPI, because the chlorophyll fluorescence of the former is too dim. Epifluorometric bacteria counts, therefore, provide an estimate of total bacteria that overstates (by about 31%) the importance of heterotrophic bacteria. On the other hand, bacteria stained with DAPI were reported to be underestimated (by about 26%) compared to those stained with acridine orange (AO) (Suzuki et al., 1993). Considering these two points, the positive and negative bias in the present study seem to be balanced. However, since microscopy is the routine method for the assessment of microorganisms, and since DAPI is preferred to AO because DAPI stained cells are more clearly visible and longer lasting, then it is necessary to directly compare different methods

Table 4. Carbon biomass ($< mu$g C l^{-1}) for total phytoplankton assemblage with its size-fractions, cyanobacteria, and heterotrophic bacteria.

Station No.	Total	Net	Nano	Pico	Cyano bacteria	Hetero bacteria	Heterotropher/ Total phytoplankton
1	10.78	–	–	–	4.15	13.00	1.20
2	12.46	3.85	5.11	3.50	1.27	9.58	0.76
3	7.21	0.49	2.59	4.13	1.49	10.48	1.45
4	5.18	0.56	2.03	2.59	0.72	6.68	1.29
5	3.36	0.49	1.54	1.33	0.05	5.68	1.69
6	4.83	0.42	2.59	1.82	0.19	6.90	1.42
7	5.53	0.35	2.66	2.52	0.20	6.88	1.24
8	5.39	0.42	2.52	2.45	0.08	6.52	1.21
9	5.53	0.49	2.66	2.38	0.03	5.72	1.03
10	4.69	0.42	2.10	2.17	0.00	5.70	1.21
11	7.35	0.07	3.78	3.57	0.17	8.94	1.21
12	8.89	0.98	4.06	4.55	0.47	10.12	1.13
Mean	6.76	0.77	2.87	2.81	0.73	8.02	1.24
SD	2.70	1.04	1.03	0.99	1.18	2.36	0.22

involved in fluorescence microscopy techniques. For a better understanding of the structures and functions of microplanktonic communities in oceanic waters, scientists need to conduct further studies on the smallest autotrophic prokaryotes (*Prochlorococcus*, 0.6 μm) and eukaryotes (*Ostreococcus tauri*, 0.70–0.97 μm; Countiers et al., 1994) by the application of flowcytometry.

Conclusions

From this study, the following conclusions can be drawn:

1. The standing biomasses of phytoplankton, cyanobacteria and heterotrophic bacteria were relatively high in the equatorial area and the offshore area, but quite low in the oligotrophic area. Vertical profiles of Chl.a, cyanobacteria and heterotrophic bacteria peaked in the upper layer of the euphotic zone (<50 m) in the equatorial area, but at the bottom layer of the euphotic zone (around 100 m) in oligotrophic waters.
2. The total amount of chlorophyll a was mainly contributed by nanoplankton and picoplankton; netplankton was responsible for a very small part (only about 10%). More than half of the >10 μm and about one third of the 0.2–1 μm pigment-containing particulate organic matter were likely to be non-living particles whereas most of the 1–10 μm pigment-containing particles were phytoplankton.
3. Except for the equatorial area, the primary production in the open ocean seemed to be limited mainly by nutrient availability. Correspondences of microorganisms to NO_3-N were more significant in larger fractions, but the opposite was true in correspondences of smaller fractions to NH_4-N.
4. Cyanobacteria were the most variable organisms and seemed not to be the principle component of the picoplankton as previously expected. Heterotrophic bacteria were the most stable organisms in oceanic waters, but they were not always the dominant microorganisms. The carbon biomass ratio of heterotrophs to phytoplankton was less than 1 in the equatorial eutrophic area and greater than 1 in other oligotrophic waters.

Acknowledgments

We are greatly indebted to Professor I. Koike for his valuable suggestions and offering the use of his facilities both in the lab and in the field. We thank the KH-93-4-cruise scientists especially Dr K. Furuya and Dr T. Saino for their kind assistance in the field. We are grateful to Mr T. Usui for his help in analyzing the nutrients. We also thank the captain and crew of Hokaho Maru for their assistance in the field.

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