

Size structures of microplankton biomass and production in Jiaozhou Bay, China

Nian-Zhi Jiao and Rong Wang

Institute of Oceanology, Academia Sinica, Qingdao 266071, China

Abstract. Seasonal investigations of size-fractionated biomass and production were carried out from February 1992 to May 1993 in Jiaozhou Bay, China. Microplankton assemblages were separated into three fractions: pico- (0.7–2 μm), nano- (2–20 μm) and netplankton (20–200 μm). The biomass was measured as chlorophyll *a* (Chl *a*), particulate organic carbon (POC) and particulate organic nitrogen (PON). The production was determined by ^{14}C and ^{15}N tracer techniques. The seasonal patterns in biomass, though variable, were characterized by higher values in spring and lower values in autumn and summer (for Chl *a* only). The seasonal patterns in production, on the other hand, were more clear with higher values occurring in summer and spring, and lower values occurring in autumn and winter. Averaged over the whole study period, the respective proportions of total biomass accounted for by net-, nano- and picoplankton were 26, 45 and 29% for Chl *a*, 32, 33 and 35% for POC, and 26, 32 and 42% for PON. The contributions to total primary production by net-, nano- and picoplankton were 31, 35 and 34%, respectively. The respective proportions of total $\text{NH}_4^+\text{-N}$ uptake accounted for by net-, nano- and picoplankton were 28, 33 and 39% in the daytime, and 10, 29 and 61% at night. The respective contributions to total $\text{NO}_3^-\text{-N}$ uptake by net-, nano- and picoplankton were 37, 40 and 23% in the daytime, and 13, 23 and 64% at night. Some comprehensive ratios, including C/N biomass ratio, Chl *a*/C ratio, C uptake/Chl *a* ratio, C:N uptake ratio and the *f*-ratio, were also calculated size separately, and their biological and ecological meanings are discussed.

Introduction

The discovery of picoplankton (Johnson and Sieburth, 1979; Waterbury *et al.*, 1979), followed by many investigations associated with its distribution in coastal (Paasche, 1988; Raimbault *et al.*, 1988) and oceanic waters [see a review by Krupatkin (1990)], have modified the general ideas of microplankton community structure. Further studies revealed that different size fractions (net-, nano- and picoplankton) have different specific ecological properties in photosynthesis (Herbland *et al.*, 1985; Chavez, 1989), nutrient uptake (Wheeler and Kirchman, 1986; Glibert, 1988; Probyn *et al.*, 1990; Chang *et al.*, 1992), energy flow model (McManus, 1991; Moloney and Field, 1991) and sediment flux (Michaels and Silver, 1988). Size-fractionated measurements of biomass and production have become an important means to understand the structure and functioning of marine ecosystems and the biogeochemical cycling of biogenic elements.

The present study was aimed at describing the size structure of plankton biomass measured as chlorophyll *a* (Chl *a*), particulate organic carbon (POC) and particulate organic nitrogen (PON), and production based on both carbon (C) and nitrogen (N) assimilation in a typical semi-closed temperate bight in China—the Jiaozhou Bay. We expected to obtain some information useful in understanding neritic ecosystems along the coastal zone.

Method

Study area and study period

Jiaozhou Bay, located in $35^{\circ}55'–36^{\circ}18'N$, $120^{\circ}05'–125^{\circ}25'E$, is a typical semi-closed temperate bay with an area of 423 km^2 and a mean depth of $\sim 7 \text{ m}$, and is characterized by high nutrient input from the cities of Qingdao, Jiaonan and Jiaozhou distributed around it. It is a field observation station of the Chinese Ecological Research Network (CERN).

The study areas for the present research work were the CERN's investigation sites 2, 5, 7, 9 and 10, but the most comprehensive experiments were conducted only at Station 5, which is located in the center of the Bay (Figure 1).

Field investigations were conducted in February, May, August and November 1992, and February and May 1993. These months were typical representatives of the local seasons.

Sampling

Water samples for the determination of Chl *a*, POC, PON and nitrogen uptake were collected with a 5 l Niskin bottle (General Oceanics) from near-surface water (0–0.5 m) and the bottom of the euphotic zone determined with a submarine photometer as the depth with 0.1% of surface incident light intensity (I_0). In case the 0.1% light level was not above the water bottom (e.g. Station 2 in winter), the bottom samples were taken from 0.5 m above the water bottom. Water samples for the determination of simulated *in situ* primary production were collected from the depth of 100% I_0 , 50% I_0 , 30% I_0 , 15% I_0 , 5% I_0 and

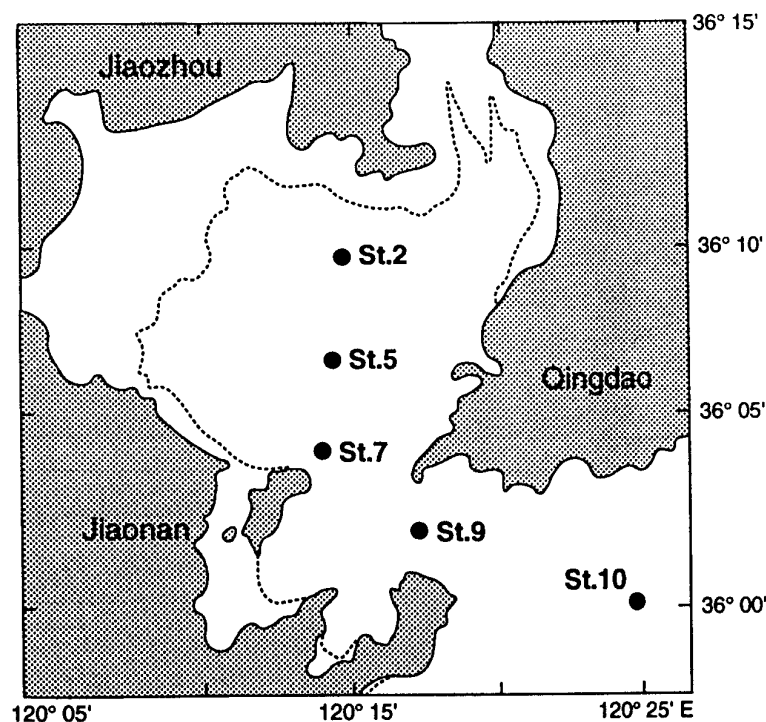


Fig. 1. The location of Jiaozhou Bay and study sites.

1% I_0 . All samples were pre-filtered through a 200 μm nylon mesh to remove larger zooplankton assemblages which might have noticeable influences on smaller populations in experiments. Duplicate samples were used for each measurement.

Size fractionation

The separations of the microorganisms were performed by filtering them through a 2 μm Nuclepore membrane (maximum filtration amount was 1 l per 47 mm diameter filter at a gentle vacuum of <0.03 MPa), and through a 20 μm plankton screen (at gravity pressure) before being filtered onto a GF/F filter with a pore diameter of ~ 0.7 μm . Consecutively subtracting the fraction <2 μm and the fraction <20 μm from the total (<200 μm) yielded the respective 0.7–2, 2–20 and 20–200 μm fractions as pico-, nano- and netplankton.

Determination of concentrations of Chl a , NO_3^- -N, NH_4^+ -N, POC and PON

Chl a content was measured by the fluorometric technique using a Turner Designs model-10 fluorometer which was calibrated with commercial Chl a (Sigma) according to the procedure described by Parsons *et al.* (1984). Concentrations of NO_3^- -N and NH_4^+ -N were determined with a Technicon II autoanalyzer by the cadmium–copper reduction method and hypochlorite oxidation method, respectively (Parsons *et al.*, 1984). POC and PON were determined simultaneously with a 240-E element autoanalyzer.

^{14}C tracer experiment and ^{14}C isotope determination

The ^{14}C uptake incubator was a 250 ml colorless-glass bottle encased in a white cloth bag that acts as a neutral density filter, reducing the light intensity to the same level as that occurring at the depth from which the sample was collected. To each bottle were added 2 μCi of $\text{NaH}^{14}\text{CO}_3$ and the samples were incubated under natural sunlight radiation in an on-deck seawater-cooled plexiglass trough for 4–6 h with occasional rocking. Duplicate incubations were carried out for each sample.

After incubation, samples were size separately filtered onto a Whatman GF/F filter as soon as possible, then the particulate samples on the filters were fumigated with concentrated hydrochloric acid for 8–10 min and, finally, the organic ^{14}C was counted using a Beckman LS-9800 Liquid Scintillation Counter with the external standard channel ratio method for quench calibration. The counting efficiency was $\sim 90\%$ and the ^{14}C uptake was calculated according to Parsons *et al.* (1984).

^{15}N tracer experiment and ^{15}N isotope determination

Uptakes of NH_4^+ -N and NO_3^- -N were measured by the ^{15}N tracer method. Aliquots (1000 ml) of the water sample were used in the incubation, 95.44 atom% enriched $\text{Na}^{15}\text{NO}_3$ and 95.26 atom% enriched $(^{15}\text{NH}_4)_2\text{SO}_4$ were added at trace concentrations of $\sim 10\%$ of the ambient nitrogen concentrations;

incubation was conducted both in the daytime and at night. The procedures were similar to that for the ^{14}C incubation experiment. Incubated water samples were filtered on pre-combusted (500°C , 6 h) GF/F filters and rinsed with 80–100 ml of 30–31‰ NaCl solution (the local salinity). Filter samples were kept in a refrigerator until analysis.

^{15}N abundance determination was performed with a ST-MS-88 mass spectrometer by the Ion-Mass-Spectrometry (IMS) method following the procedures described by Jiao *et al.* (1993). The uptake rate was calculated according to the formulation of Grunseich *et al.* (1980).

Terms and expression

The C/N biomass ratio and the Chl *a*/C ratio were calculated by weight. The assimilation number (AN) was expressed as a photosynthetic rate per unit Chl *a* per hour ($\text{mg C mg}^{-1} \text{ Chl } a \text{ h}^{-1}$). The C:N uptake ratio was calculated as the ratio of the photosynthetic rate to the sum of NO_3^- -N and NH_4^+ -N uptake rates. The proportion of NO_3^- -N uptake in the sum of NO_3^- -N uptake and NH_4^+ -N uptake is termed the *f*-ratio (Eppley and Peterson, 1979), and often used as an index of the proportion of new production in total primary production. In the case of this study, there were large nutrient (including NH_4^+ and NO_3^-) inputs from the surroundings. Therefore the ratio no longer had this meaning, but was used as an indicator of the differential use of NO_3^- and NH_4^+ by different plankton fractions. Here we symbolize the ratio as the *f'*-ratio.

Results

Temperature and nitrogen nutrients

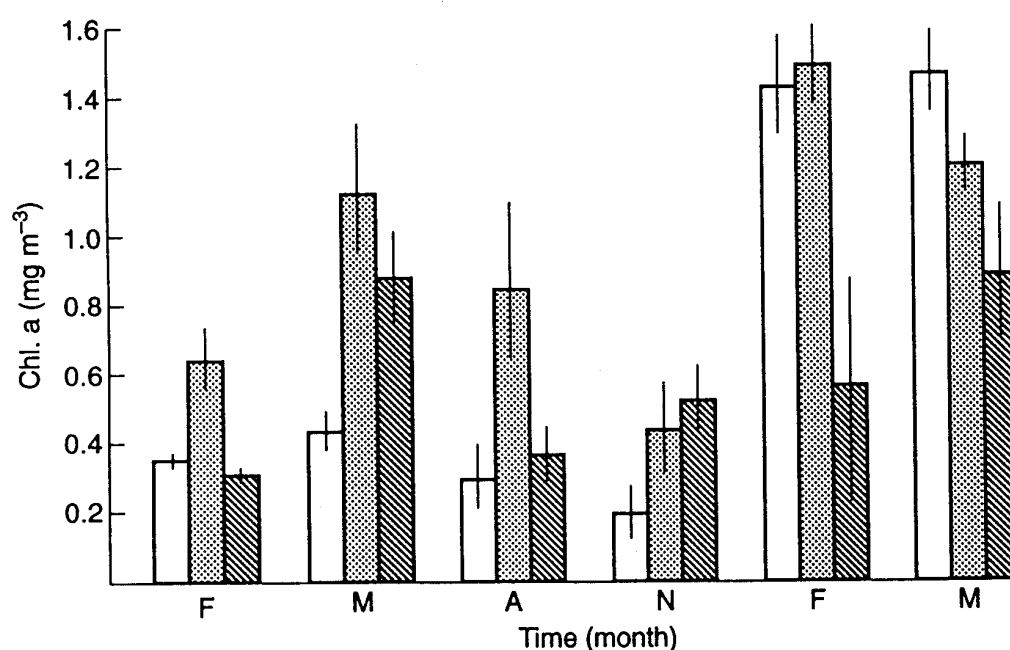
Because of the shallow water column and wind-driven advection, no distinct differences in both temperature and nutrients between the surface layer and bottom layer were recorded in most cases and at most investigation sites (including Station 5). Temporal fluctuations, however, were noticeable. During the study period, seawater temperature varied from 4.3°C in winter to 27.0°C in summer; NH_4^+ -N ranged from 3.78 mmol m^{-3} in summer to $11.07 \text{ mmol m}^{-3}$ in winter, and NO_3^- -N ranged from 0.56 mmol m^{-3} in summer to 1.43 mmol m^{-3} in winter. Values of the ratio of NO_3^- -N to the sum of NO_3^- -N and NH_4^+ -N were very low, ranging from 0.086 to 0.165, indicating that NH_4^+ -N dominated throughout all seasons in Jiaozhou Bay (Table I).

Chlorophyll a

Size-fractionated Chl *a* measurements were conducted at Stations 2, 5, 7, 9 and 10 on all the cruises. Averaged over five investigation sites, net-Chl *a* ranged from 0.21 ± 0.19 to $1.49 \pm 1.5 \text{ mg Chl } a \text{ m}^{-3}$, with high values occurring in spring and winter, and low values occurring in autumn and summer. Nano-Chl *a* ranged from 0.46 ± 0.15 to $1.5 \pm 0.87 \text{ mg Chl } a \text{ m}^{-3}$, following the same trend as net-Chl *a*. Pico-Chl *a* ranged from 0.35 ± 0.08 to $0.90 \pm 0.34 \text{ mg Chl } a \text{ m}^{-3}$, with maximum values in spring and lower values in other seasons (Figure 2). The

Table 1. Seawater temperature and ambient nitrogen concentration at the CERN's Station 5 in Jiaozhou Bay

Time	Temperature (°C)	NO ₃ ⁻ -N (mmol m ⁻³)	NH ₄ ⁺ -N (mmol m ⁻³)	NO ₃ ⁻ -N / (NO ₃ ⁻ -N + NH ₄ ⁺ -N)
Feb. 1992	4.3	1.06	11.07	0.088
May 1992	12.0	0.63	3.85	0.141
Aug. 1992	27.0	0.56	3.78	0.129
Nov. 1992	13.5	0.93	6.96	0.118
Feb. 1993	4.0	1.43	7.25	0.165
May 1993	14.5	1.02	10.80	0.086
Mean ± SD		0.94 ± 0.32	7.33 ± 3.25	0.12 ± 0.03

**Fig. 2.** Seasonal variations of net- (white), nano- (hatched) and pico-Chl *a* (black) concentrations averaged over the CERN's Stations 2, 5, 7, 9 and 10 in Jiaozhou Bay. The error bars represent SD.

relative contributions to total Chl *a* biomass by net-, nano- and picoplankton were 15 ± 9 – $41 \pm 6\%$ with a mean value of $26 \pm 10\%$, 39 ± 12 – $56 \pm 4\%$ with a mean value of $45 \pm 6\%$, and 18 ± 3 – $45 \pm 11\%$ with a mean value of $29 \pm 9\%$, respectively. Nanoplankton dominated in most seasons, followed by picoplankton. Netplankton, on the other hand, accounted for only a small proportion during 1992, but increased drastically in early 1993 when the total Chl *a* exceeded $5 \text{ mg Chl } a \text{ m}^{-3}$ at some investigation sites and a Chl *a* bloom was apparently observed.

POC and PON

Seasonal variations of POC and PON followed a more or less similar trend to Chl *a* in all seasons except summer (Figure 3). Total POC was shared almost equally by net- (28–38%), nano- (25–42%) and picoplankton (26–47%). For

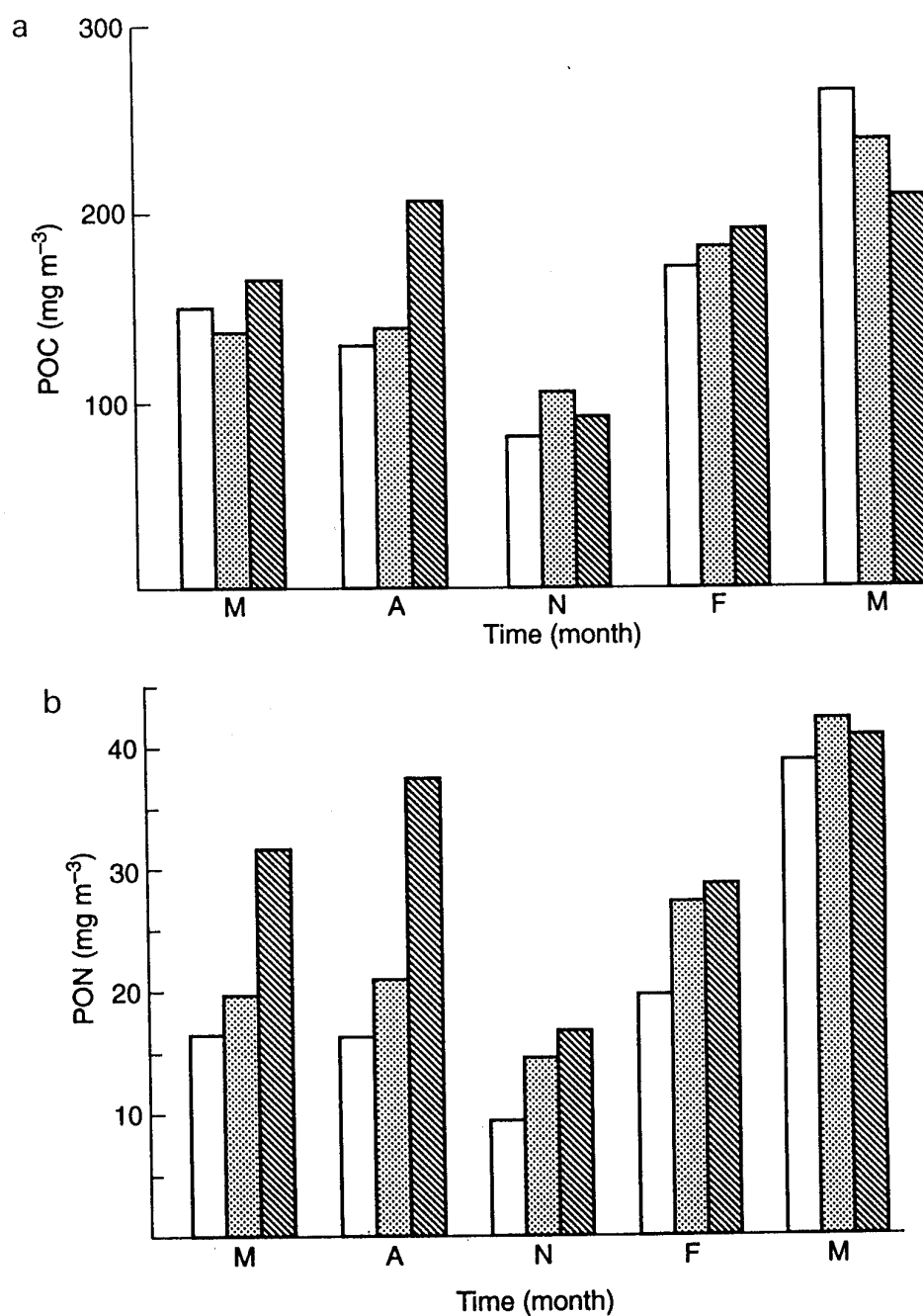


Fig. 3. Seasonal variations of POC (a) and PON (b) in net- (white), nano- (hatched) and picoplankton (black) at the CERN's Station 5 (open) and Station 10 (solid) in Jiaozhou Bay.

PON, however, the respective percentages accounted for by the three fractions were 22–32, 28–39 and 34–50, showing an apparent order of pico-PON > nano-PON > net-PON.

*The C/N ratio and Chl *a*/C ratio*

Although there was no apparent seasonal pattern in the C/N ratio, significant differences among the three fractions were observed, with the net-C/N ratio > nano-C/N ratio > pico-C/N ratio (Table II).

The dynamics of the Chl *a* content per unit of carbon in the three size fractions

Table II. Size-fractionated C/N weight ratio of microorganisms at the CERN's Stations 5 and 10 in Jiaozhou Bay

Time	Site	Total	Netplankton	Nanoplankton	Picoplankton
May 1992	5	6.60	9.53	6.99	4.90
Aug. 1992	5	6.50	8.40	6.79	5.52
	10	5.66	7.79	6.09	4.43
Nov. 1992	5	7.26	8.69	8.11	5.65
Feb. 1993	5	6.83	8.42	6.47	6.08
	10	6.55	8.66	6.24	5.68
May 1993	5	5.67	6.67	5.58	4.85
	10	6.60	9.53	6.99	4.90
Mean \pm SD		6.45 \pm 0.55	8.46 \pm 0.93	6.65 \pm 0.76	5.25 \pm 0.56

Table III. Size-fractionated Chl *a*/C ratio ($\times 10^{-2}$) of microorganisms at the CERN's Station 5 in Jiaozhou Bay

Time	Total	Netplankton	Nanoplankton	Picoplankton
May 1992	0.950	0.595	0.751	0.705
Aug. 1992	0.190	0.165	0.443	0.151
Nov. 1992	0.420	0.264	0.604	0.337
Feb. 1992	0.454	0.724	0.516	0.159
May 1993	0.451	0.366	0.466	0.421
Mean \pm SD	0.430 \pm 0.152	0.423 \pm 0.232	0.556 \pm 0.125	0.354 \pm 0.220

had a general character that the lowest values occurred in summer, but the fluctuation of nanoplankton was much smaller than that of the other two fractions. The averaged Chl *a*/C ratio over the study period was highest in nano-, lowest in pico- and medium in netplankton (Table III).

Primary production

Total primary production during the study period varied from 247 mg C m⁻² day⁻¹ in November 1992 to 714 mg C m⁻² day⁻¹ in May 1993. Two peaks occurred in summer (1992) and spring (1993) (Figure 4). The contributions of the three cell-size fractions to the total production differed during the period. In February, May, August and November 1992, the primary contributor was pico- (38–45%) followed by nano- (30–37%) and netplankton (18–30%). In February and May 1993, on the other hand, netplankton became the primary contributor accounting for 42–44%, picoplankton contributed the least being responsible for ~21%, and nanoplankton were intermediate at 35–37%. Mean contributions, averaged over the whole period, by net-, nano- and picoplankton were 31 \pm 10%, 35 \pm 3% and 34 \pm 10%, respectively. It was evident from the SD values that the contribution of the nanoplankton fraction to the total community production was rather constant along the temporal axis, but those of the net- and picoplankton fractions were more variable.

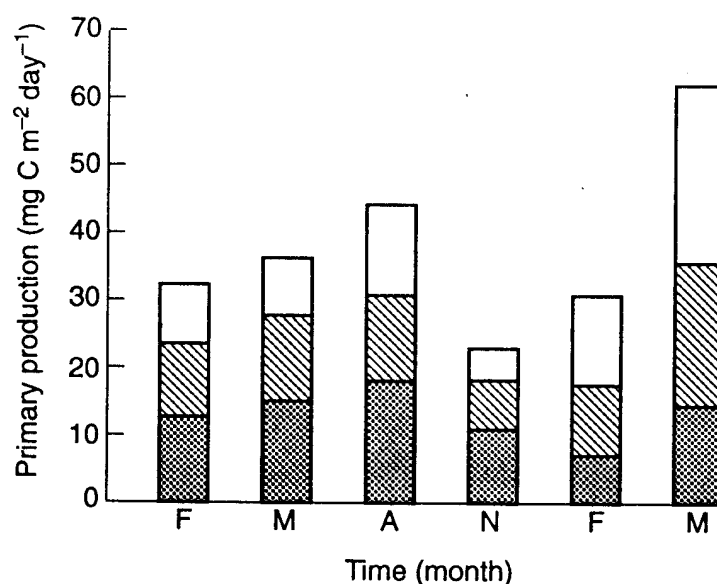


Fig. 4. Depth-integrated primary production for net- (white), nano- (hatched) and picoplankton (black) at the CERN's Station 5 in Jiaozhou Bay.

Table IV. Size-fractionated assimilation number ($\text{mg C mg}^{-1} \text{ Chl } a \text{ h}^{-1}$) averaged over the euphotic zone at CERN's Station 5 in Jiaozhou Bay

Time	Total	Netplankton	Nanoplankton	Picoplankton
Feb. 1992	2.09	2.30	1.51	2.52
May 1992	1.23	1.53	1.15	1.16
Aug. 1992	3.17	4.55	2.03	3.93
Nov. 1992	1.65	1.65	1.07	2.92
Feb. 1993	1.17	1.02	1.01	2.35
May 1993	1.68	1.91	1.77	1.26
Mean \pm SD	1.83 \pm 0.74	2.16 \pm 1.25	1.42 \pm 0.42	2.36 \pm 1.04

The assimilation number

Netplankton had a higher mean AN value of 2.16 ± 1.25 with a wide range of 1.02–4.55 $\text{mg C mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ than nanoplankton which had a mean value of 1.42 ± 0.42 spanning a range of 1.01–2.03 $\text{mg C mg}^{-1} \text{ Chl } a \text{ h}^{-1}$. Having a relatively lower Chl *a* content, picoplankton had a highest mean AN value of 2.36 ± 1.04 , with fluctuations of between 1.16 and 3.93 $\text{mg C mg}^{-1} \text{ Chl } a \text{ h}^{-1}$ (Table IV). These results suggested that the potential production capacity for the three fraction was in the order pico- > net- > nanoplankton. What all the three fractions had in common was that the highest values of AN occurred in summer, indicating that a relationship between production capacity and temperature existed in all the different size fractions.

Nitrogen uptake

Uptake of NH_4^+ -N was measured both in the daytime and at night. Total uptake of NH_4^+ -N was higher in summer and spring, and lower in winter and autumn

(Figure 5). The percentages accounted for in the total NH_4^+ -N uptake by net-, nano- and picoplankton in the light were 17–47, 29–42 and 18–64% with respective means of 28 ± 13 , 33 ± 6 and $39 \pm 15\%$. The uptake order for the three fractions was pico- > nano- > netplankton. This trend was further strengthened in dark conditions where the average percentages of net-, nano- and picoplankton were 10 ± 4 , 29 ± 9 and $61 \pm 12\%$, respectively.

The dynamics of the total uptake of NO_3^- -N are similar to that of NH_4^+ -N (Figure 6). The partial uptakes of NO_3^- -N by the three fractions, however, were not in the same order as those for NH_4^+ -N. In the daytime, uptake percentages for net- and nanoplankton were quite similar (37 ± 10 and $40 \pm 9\%$), and that for picoplankton was distinctly lower ($23 \pm 13\%$). At night, however, the total uptake was contributed mainly by picoplankton ($64 \pm 15\%$), and net- and nanoplankton accounted for only 13 ± 10 and $23 \pm 6\%$, respectively.

The f' -ratio

The f' -ratio fluctuated during the study period (Table V). In the daytime, the f' -ratio in net- and nanoplankton varied in a similar pattern with the highest values in summer and the lowest values in winter. On the other hand, picoplankton followed a seasonal trend almost in the opposite direction to the largest fractions. Overall, average values of the f' -ratio in different size fractions were in the order net- > nano- > picoplankton.

Values of the f' -ratio were much smaller under dark conditions than under light conditions. The differences between dark and light conditions were largest in nanoplankton and smallest in picoplankton, indicating that uptake of NO_3^- -N in picoplankton was less light dependent than in net- and nanoplankton.

The C:N uptake ratio

The C:N uptake ratio at the surface layer of Station 5 showed a distinct seasonal pattern, in which the lowest values occurred in productive seasons such as summer 1992 and spring 1993 (apart from netplankton). Average values for net-, nano- and picoplankton were 9.38 ± 2.39 , 9.02 ± 2.05 and 8.12 ± 2.24 , respectively (Table VI).

Discussion

Size-fractionated biomass

Chl *a*, POC and PON are often used as biomass indices for microorganisms in the sea. As Chl *a* is not contained in heterotrophic organisms and non-living particles, it is a unique biomass index for phytoplankton, but due to the differences in Chl *a* content in terms of per unit of carbon among species and seasons, it does not necessarily represent exact biomass in weight. On the other hand, the direct indices of biomass (POC and PON) also have some disadvantages of not being able to distinguish autotrophic and heterotrophic organisms. Therefore, those indices should be used simultaneously and some combined indices, such as the C/N ratio and Chl *a*/C ratio, can be worked out to

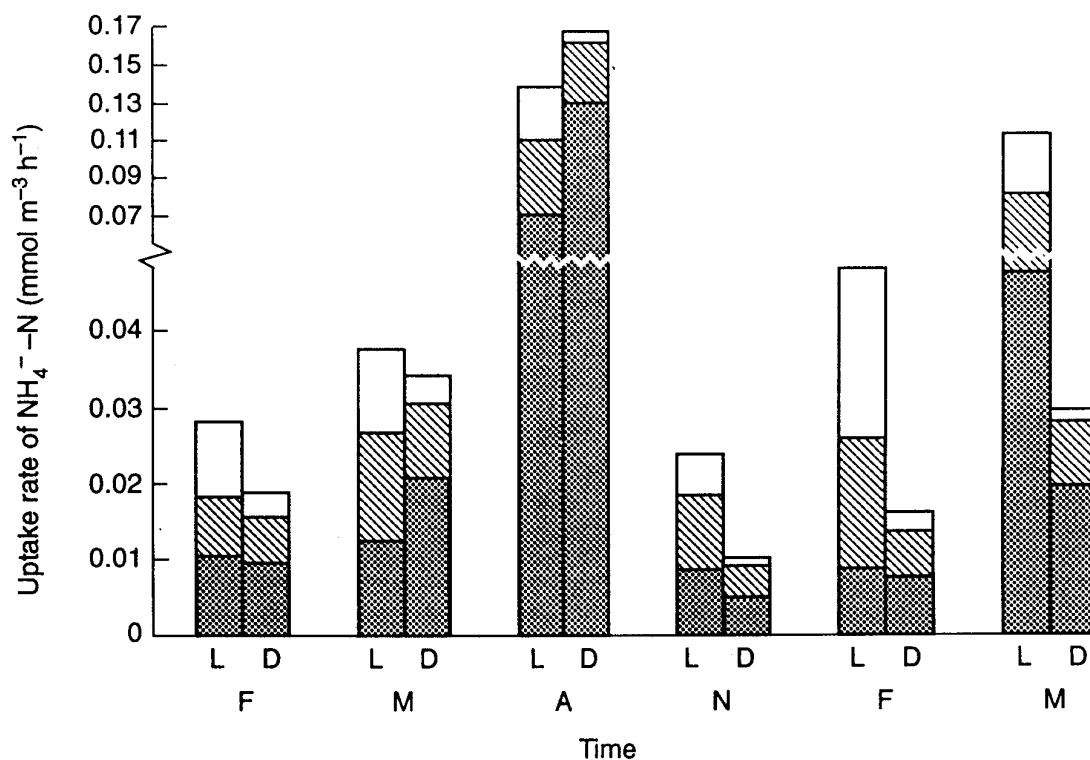


Fig. 5. NH_4^+-N uptake by net- (white), nano- (hatched) and picoplankton (black) at the CERN's Station 5 in Jiaozhou Bay. L, in the daytime; D, at night.

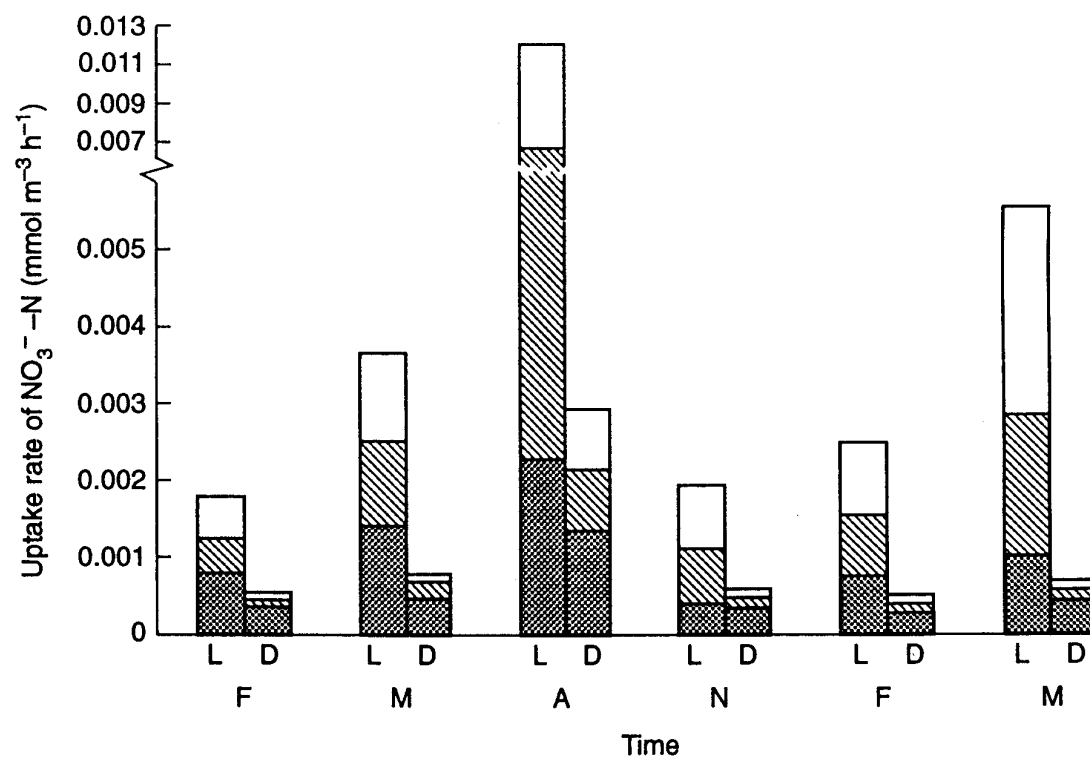


Fig. 6. NO_3^--N uptake by net- (white), nano- (hatched) and picoplankton (black) at the CERN's Station 5 in Jiaozhou Bay. L, in the daytime; D, at night.

Table V. Dynamics of the f' -ratio (%) in different size fractions of microplankton at CERN's Station 5 in Jiaozhou Bay

Time	Total		Netplankton		Nanoplankton		Picoplankton	
	L	D	L	D	L	D	L	D
Feb. 1992	6.04	2.56	4.76	0.67	7.89	1.32	5.98	3.85
May 1992	8.87	2.23	9.09	3.25	7.89	2.44	9.72	2.04
Aug. 1992	8.52	1.69	17.30	7.22	15.37	2.23	3.10	1.14
Nov. 1992	7.69	5.66	11.76	5.02	8.26	2.01	3.61	4.01
Feb. 1993	5.04	3.75	4.56	2.89	5.03	1.51	6.25	3.30
May 1993	4.88	2.41	8.52	3.40	5.51	2.30	2.00	2.75
Mean \pm SD	6.84 \pm 1.75	3.05 \pm 1.44	9.33 \pm 4.77	3.74 \pm 2.20	8.33 \pm 3.71	1.97 \pm 0.45	5.11 \pm 2.80	2.85 \pm 1.10

Table VI. Dynamics of the C:N uptake ratio in different size fractions of microplankton at CERN's Station 5 in Jiaozhou Bay

Time	Total		Netplankton		Nanoplankton		Picoplankton	
	L	D	L	D	L	D	L	D
Feb. 1992	12.77	11.40	14.57	12.33	14.57	12.33	14.57	12.33
May 1992	8.86	9.49	9.51	7.35	9.51	7.35	9.51	7.35
Aug. 1992	6.49	6.99	6.40	6.17	6.40	6.17	6.40	6.17
Nov. 1992	8.95	6.29	7.45	7.22	7.45	7.22	7.45	7.22
Feb. 1993	7.68	9.83	8.94	8.82	8.94	8.82	8.94	8.82
May 1993	8.09	12.29	7.23	6.83	7.23	6.83	7.23	6.83
Mean \pm SD	8.81 \pm 2.14	9.38 \pm 2.39	9.02 \pm 2.95	8.12 \pm 2.24	9.02 \pm 2.95	8.12 \pm 2.24	9.02 \pm 2.95	8.12 \pm 2.24

give a better understanding of the composition of microplankton communities qualitatively and quantitatively.

In the present study, size-fractionated biomass orders were nano- > net- > picoplankton in terms of Chl *a*, and pico- > nano- > netplankton in terms of POC and PON. It can be deduced by comparing the orders with each other that there might be more heterotrophic organisms, such as bacteria, as well as detritus in the smallest fraction (picoplankton) than in larger fractions. Summarizing the three indices, the distributions of total biomass in net-, nano- and picoplankton fractions were about 28, 38 and 34%, respectively. These proportions were comparable with previous results from coastal or near-shore regions (Probyn *et al.*, 1990; Chang *et al.*, 1992).

From the seasonal variations of all three biomass indices (Figures 2 and 3, and Table III), it was evident that there was a biomass bloom in the period of February–May 1993. From the percentage distributions, it can be inferred that the biomass increase in early 1993 was caused mainly by netplankton. This was illustrated more clearly by plotting the size-fractionated Chl *a* versus the total Chl *a* (Figure 7). In Figure 7, when total Chl *a* exceeds 3 mg m^{-3} , the relationship between pico-Chl *a* and total-Chl *a* becomes non-linear, but nano- and especially net-Chl *a* still maintain a clear linear correlation with total-Chl *a*. These results are similar to the conclusion of Raimbault *et al.* (1988) that large Chl *a* stocks are hardly formed by small plankton but by netplankton, and noticeable blooms of pico- and nanoplankton seldom occur under natural conditions in productive temperate waters. The eutrophication of marine environments, which results in large Chl *a* biomasses, should result in blooms of netplankton. Compared with environmental changes (e.g. temperature), the

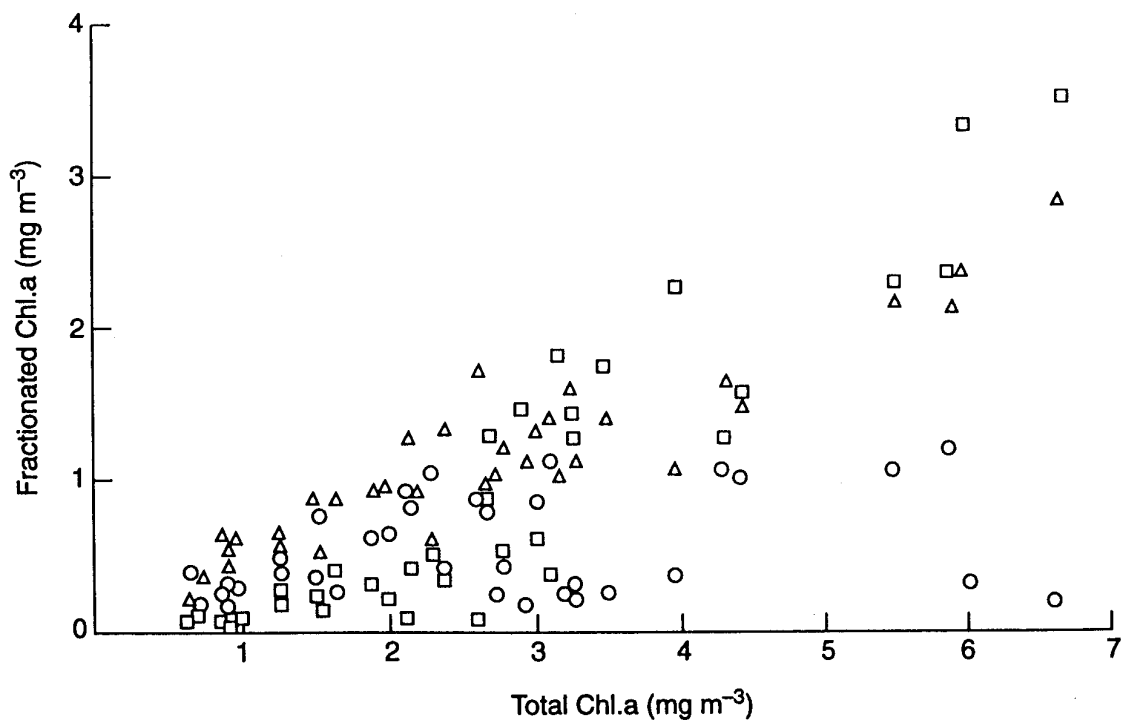


Fig. 7. Relationships between total Chl *a* stocks and net- (\square), nano- (\triangle) and pico- (\circ) Chl *a* fractions.

variations in biological measurements among seasons were relatively low. This was mainly due to the efficient species succession (Y.-H.Gao and N.-Z.Jiao, unpublished, 1994).

Size-fractionated primary production

Previous studies on size-fractionated primary production revealed that the smaller fractions are more productive than the large ones (Larsson and Hagstrom, 1982; Paasche, 1988; Hall and Vincent, 1990). In oligotrophic environments, picoplankton is responsible for up to 70% of total Chl *a* and up to 80% of total primary production (Krupatkina, 1990), i.e. compared with the given proportion in the total biomass, picoplankton accounts for a greater proportion of total production. In the present study, the overall average contribution of netplankton to the total was ~30%, and the rest was shared by the two smaller fractions. Apart from the bloom period of February–May 1993, however, the contribution of picoplankton was much greater (~40%). A similar situation was also reported by Larsson and Hagstrom (1982). The spring bloom resulted from an increase in the biomass of the larger fractions, especially netplankton. Although size-fractionated primary production may be confounded by possible grazing, these size differences characterize the differences between the coastal and oceanic waters in both biomass and production.

Size-fractionated nitrogen uptake

As the primary nutrient limiting primary production, nitrogen metabolism has always been one of the study subjects for ecologists. The present study on size-fractionated nitrogen uptake added more interesting information on this aspect.

Under light conditions, NH_4^+ -N was taken up mainly by the smaller fractions and NO_3^- -N was taken up mainly by the larger fractions. Under dark conditions, on the other hand, both NH_4^+ -N and NO_3^- -N were taken up mainly by the smaller fractions. It is thus evident that the larger fractions were more light dependent than the smaller ones and that the preference for NH_4^+ -N compared with NO_3^- -N of smaller fractions was stronger than that of larger ones. Malone (1980) proposed a hypothesis that net-plankton utilize primarily new N as NO_3^- -N and smaller fractions mainly use regenerated N as NH_4^+ -N. This was supported by the fact that netplankton blooms often developed in response to NO_3^- -N input, and other study results that pico- and nanoplankton utilize more reduced N than netplankton (Probyn, 1985; Koike *et al.*, 1986), but exceptions are common (Furnas, 1983; Harrison and Wood, 1988). According to the experimental results of this study and another study (Jiao, 1993), we are of the opinion that all three fractions prefer NH_4^+ -N to NO_3^- -N but the extent of the preference is weaker in netplankton than in the other two fractions. Thus, if NO_3^- -N and NH_4^+ -N are supplied equally, NH_4^+ -N should be used prior to NO_3^- -N by all three fractions, but netplankton will be responsible for a larger proportion of the uptake of NO_3^- -N than nano- or picoplankton.

Comparing light uptake with dark uptake of different nitrogen sources by different size categories, it was evident that NO_3^- -N uptake was more light

dependent than NH_4^+ -N uptake, and larger fractions were more light dependent than smaller ones. These conclusions were also drawn from our and other researchers' results (Probyn *et al.*, 1990; Jiao and Wang, 1993).

Biological and chemical characteristics in size-fractionated biomass

As different kinds of marine organisms have different specific element compositions, biological and chemical ratios such as C/N, Chl *a*/C are useful for distinguishing different components in microplankton communities. The Redfield ratio is widely used as a normal C/N ratio for phytoplankton and is 6.6 in atom and 5.7 in weight (Redfield *et al.*, 1963). However, values of the C/N ratio reported in the literature were often higher, e.g. 5.1–10.5 (Parsons *et al.*, 1961) and 6–10 (Wheeler, 1983). Values observed in the present study ranging from 4.5 to 9.5 were similar to those mentioned above, but the significant differences in C/N ratio among different fractions (Table II) implied the differences in their biological composition. As the C/N ratios in bacteria and protozoans are relatively small, ranging from 3 to 6 (Finlay and Uhlig, 1981; Wheeler, 1983; Goldman *et al.*, 1987, Lee and Fuhrman, 1987), it can be deduced from the size-fractionated C/N ratio (Table II) that the small fractions contained many non-phytoplankton fractions such as bacteria and protozoans.

If the above deductions were correct, some other properties could be deduced from the Chl *a*/C ratio. Apart from the picoplankton which might contain a large quantity of heterotrophic organisms as well as non-living detritus, the carbon-specific Chl *a* content seemed to be in inverse proportion to the phytoplankton cell size. This inference is consistent with a review on picoplankton by Krupatkina (1990).

Physiological and ecological characteristics in size-fractionated production

The photosynthetic efficiency of phytoplankton can be expressed by the AN. It is generally considered that the smaller the cell size, the more efficient the photosynthesis (Krupatkina, 1990). In the present study, the smallest fraction picoplankton had, in fact, the highest mean AN, but nanoplankton, on the other hand, had a smaller AN than netplankton. Why nanoplankton had the lowest photosynthetic efficiency is beyond the scope of the discussion here, but unusual facts were also recorded in a well-conceived field experiment (Happey-Wood, 1993) in which the picoplankton even becomes the inefficient fraction. Thus, the matter seems not to be so simple and needs further inquiry into both ecological and physiological aspects (such as grazing effects, photosynthetic efficiency, etc.) associated with size fractionation.

The values of the C:N uptake ratio in net- and nanoplankton were similar, but that in picoplankton was much smaller. The higher uptake of nitrogen in proportion to carbon in picoplankton was consistent with the situation of its C/N biomass ratio, and heterotrophic bacterial uptake of nitrogen should be responsible for the majority of differences among size categories.

Comparing Figure 4 with Figures 5 and 6, differences in the extent of the seasonal variation between C, N uptakes become obvious. The effect of

temperature on uptake of N was greater than that of C, although uptake rates of both C and N were increased with increasing temperature. This pattern is identical to that for the central North Pacific Ocean (Eppley *et al.*, 1977).

The average values of the C:N uptake ratio in Jiaozhou Bay were relatively lower compared with other coastal (12.4:1) and oceanic waters (19:1) (McCarthy, 1972; Eppley *et al.*, 1977). This may be due to the high nitrogen supply in Jiaozhou Bay (Table I).

The *f*-ratio is of crucial importance for understanding the functioning of marine ecosystems. Although here the *f'*-ratio does not reflect the realities of new production, it does suggest that new production would be contributed mainly by larger fractions and regenerated production would be formed mainly by smaller fractions. Furthermore, values of the *f'*-ratio under dark conditions were much lower than under light conditions, but the decline for picoplankton was much smaller than that for net- and nanoplankton, i.e. the smallest fraction was responsible for a relatively greater proportion of total NO_3^- -N uptake at night than in the daytime. This phenomenon can be explained by the heterotrophic utilization of NO_3^- -N (Harrison, 1973; Fuhrman and Azam, 1980) which does not require light. Existing mainly in picoplankton, heterotrophic organisms make this size fraction more light independent, and more productive in terms of nitrogen. On the other hand, the complexities in the dynamics of the *f*-ratio make the prediction/estimation of new production in marine environments more difficult and give more impact to the methodology of ^{15}N tracer experiment–*f*-ratio–new production.

Values of the *f'*-ratio recorded in the present study were very low in comparison with other coastal regions, such as Chesapeake Bay with an *f*-ratio of 0.3 (McCarthy *et al.*, 1977), Carmans Estuary, NY, with an *f*-ratio of 0.51 (Carpenter and Dunham, 1985), Bedford Basin, Nova Scotia, with an *f*-ratio of 0.39 (La Roche, 1983) and Narragansett Bay, RI (Furnas, 1983). This was due to the special nutrient conditions in Jiaozhou Bay, where NH_4^+ -N was the dominating nitrogen source throughout the study period (Table I) and the ratio of NO_3^- -N to the sum of NO_3^- -N and NH_4^+ -N was only 0.096–0.165. In addition, uptake inhibition of NO_3^- -N by NH_4^+ -N was noticeable (Jiao, 1993). Therefore, the low values of the *f*-ratio are reasonable.

Acknowledgements

We thank Mr Li Chaolun and Ji Peng for their efficient assistance in the field, and leaders of Jiaozhou Bay Investigation Station (CERN) for their enthusiastic support in arrangements of the investigation cruises. This study was financially supported by the National Natural Science Foundation of China, No. 39200022.

References

- Carpenter, E.J. and Dunham, S. (1985) Nitrogen nutrient uptake, primary production, and species composition of phytoplankton in the Carmans River estuary, Long Island, N.Y. *Limnol. Oceanogr.*, **39**, 221–232.
- Chang, F.H., Vincent, W.F. and Woods, P.H. (1992) Nitrogen utilization by size-fractionated

- phytoplankton assemblages associated with an upwelling event off Westland, New Zealand. *N. Z. J. Mar. Freshwater Res.*, **26**, 287–301.
- Chavez, F.P. (1989) Size distribution of phytoplankton in the central and eastern tropical Pacific. *Global Biochem. Cycles*, **3**, 27–35.
- Eppley, R.W. and Peterson, B.J. (1979) Particulate organic matter flux and planktonic new production in the deep ocean. *Nature*, **282**, 677–680.
- Eppley, R.W., Sharp, J.H., Renger, E.H., Perry, M.J. and Harrison, W.G. (1977) Nitrogen assimilation by phytoplankton and other microorganisms in the surface waters of the central North Pacific Ocean. *Mar. Biol.*, **39**, 111–120.
- Finlay, B.J. and Uhlig, G. (1981) Calorific and carbon values of marine and freshwater Protozoan. *Helgol. Meeresunters.*, **34**, 401–412.
- Fuhrman, J. and Azam, F. (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. *Appl. Environ. Microbiol.*, **39**, 1085–1095.
- Furnas, M.J. (1983) Nitrogen dynamics in lower Narragansett Bay, Rhode Island. 1. Uptake by size-fractionated phytoplankton populations. *J. Plankton Res.*, **5**, 657–676.
- Glibert, P.M. (1988) Primary productivity and pelagic nitrogen cycling. In Blackburn, T.H. and Sørensen, J. (eds), *Nitrogen Cycling in Coastal Marine Environments*. John Wiley & Sons, pp. 3–31.
- Goldman, J.C., Caron, D.A. and Dennett, M.R. (1987) Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnol. Oceanogr.*, **32**, 1239–1252.
- Grunseich, G.S., Dugdale, R.C., Breitner, N.F. and MacIsaac, I.J. (1980) Sample conversion, mass spectrometry and calculations for N analysis of phytoplankton nutrient uptake. *Coastal Upwelling Ecosystems Analysis (CUEA) Tech. Rep.* 44.
- Harrison, W.G. (1973) Nitrate reductase activity during a dinoflagellate bloom. *Limnol. Oceanogr.*, **18**, 457–465.
- Harrison, W.G. and Wood, L.J.E. (1988) Inorganic nitrogen uptake by marine picoplankton. Evidence for size partitioning. *Limnol. Oceanogr.*, **33**, 468–475.
- Hall, J.A. and Vincent, W.F. (1990) Picoplankton of coastal upwelling system. *Mar. Biol.*, **106**, 465–471.
- Happey-Wood, C.M. (1993) Diurnal and seasonal variation in the contributions of autotrophic pico-, nano- and microplankton to the primary production of an upland lake. *J. Plankton Res.*, **15**, 125–159.
- Herbland, A., Bouteiller, A.L. and Raimbault, P. (1985) Size structure of phytoplankton biomass in the equatorial Atlantic Ocean. *Deep-Sea Res.*, **32**, 819–836.
- Jiao, N.Z. (1993) Interactions between ammonium uptake and nitrate uptake by natural phytoplankton assemblages. *Chin. J. Oceanol. Limnol.*, **11**, 97–107.
- Jiao, N.Z. and Wang, R. (1993) Size-fractionated nitrogen uptake kinetics of microplankton assemblages in Jiaozhou Bay. *Chin. J. Ecol.*, **12B**, in press (in Chinese with English abstract).
- Jiao, N.Z., Wang, R. and Huang, Q.W. (1993) Determination of ^{15}N isotope by Ion-Mass-Spectrometry method and its application to the study of new production and nitrogen cycling in marine environments. *Limnol. et Oceanol. Sin.*, **24**, 65–71 (in Chinese with English abstract).
- Johnson, P.W. and Sieburth, J. (1979) Chroococcoid cyanobacteria in the sea. An ubiquitous and diverse biomass. *Limnol. Oceanogr.*, **24**, 928–935.
- Koike, I., Holm-Hansen, O. and Biggs, D.G. (1986) Inorganic nitrogen metabolism by Antarctic phytoplankton with special reference to ammonium cycling. *Mar. Ecol. Prog. Ser.*, **30**, 105–116.
- Krupatkina, D.K. (1990) Estimates of primary production in oligotrophic waters and metabolism of picoplankton: a review. *Mar. Microb. Food Webs*, **4**, 87–102.
- La Roche, J. (1983) Ammonium regeneration: its contribution to phytoplankton nitrogen requirements in a eutrophic environment. *Mar. Biol.*, **76**, 231–240.
- Larsson, V. and Hagstrom, Å. (1982) Fractionated phytoplankton primary production, exudate release and bacterial production in a Baltic Eutrophication Gradient. *Mar. Biol.*, **67**, 57–70.
- Lee, S. and Fuhrman, J.A. (1987) Relationships between biovolume and biomass of naturally-derived marine bacterioplankton. *Appl. Environ. Microbiol.*, **53**, 1298–1303.
- McCarthy, J.J. (1972) The uptake of urea by natural populations of marine phytoplankton. *Limnol. Oceanogr.*, **17**, 738–748.
- McCarthy, J.J., Taylor, W.R. and Taft, J.L. (1977) Nitrogenous nutrition of the plankton in the Chesapeake Bay. 1. Nutrients availability and phytoplankton preferences. *Limnol. Oceanogr.*, **22**, 996–1011.
- McManus, G.B. (1991) Flow analysis of a planktonic microbial food web model. *Mar. Microb. Food Webs*, **5**, 145–160.

- Michaels, A.F. and Silver, M.W. (1988) Primary production, sinking fluxes and the microbial food web. *Deep-Sea Res.*, **35**, 473–490.
- Malone, T.C. (1980) Size-fractionated primary production of marine phytoplankton. In *Primary Productivity in the Sea. Brookhaven Symp. Biol. 31*. Plenum, pp. 301–319.
- Moloney, C.L. and Field, J.G. (1991) The size-based dynamics of plankton food webs I. A simulation model of carbon and nitrogen flows. *J. Plankton Res.*, **13**, 1003–1038.
- Paasche, E. (1988) Pelagic primary production in nearshore waters. In Blackburn, T.H. and Sørensen, J. (eds), *Nitrogen Cycling in Coastal Marine Environments*. John Wiley & Sons, pp. 33–57.
- Parsons, T.R., Stephens, K. and Strickland, J.D.H. (1961) On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Board Can.*, **18**, 1001–1016.
- Parsons, T.R., Maita, Y. and Lalli, C.M. (1984) *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, pp. 3–122.
- Probyn, T.A. (1985) Nitrogen uptake by size-fractionated phytoplankton populations in the southern Benguela upwelling system. *Mar. Ecol. Prog. Ser.*, **22**, 249–258.
- Probyn, T.A., Waldron, H.N. and James, A.G. (1990) Size-fractionated measurements of nitrogen uptake in aged upwelled waters: Implication for pelagic food webs. *Limnol. Oceanogr.*, **35**, 202–210.
- Raimbault, P., Rodier, M. and Taupier-Letage, I. (1988) Size fraction of phytoplankton in the Ligurian Sea and the Algerian Basin (Mediterranean Sea): Size distribution versus total concentration. *Mar. Microb. Food Webs*, **3**, 1–7.
- Redfield, A.C., Ketchum, B.H. and Richards, F.A. (1963) The influence of organism on the composition of seawater. In Hill, M.N. (ed.), *The Sea*. Interscience, New York, pp. 26–77.
- Waterbury, J.B., Watson, S.W., Guillard, R.R.L. and Brand, L.E. (1979) Widespread occurrence of a unicellular marine plankton cyanobacteria. *Nature*, **277**, 293–294.
- Wheeler, P.A. (1983) Phytoplankton metabolism. In Carpenter, E.J. and Capone, D.G. (eds), *Nitrogen in the Marine Environment*. Academic Press, New York, pp. 309–346.
- Wheeler, P.P. and Kirchman, D.L. (1986) Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol. Oceanogr.*, **31**, 998–1009.

Received on November 1, 1993; accepted on July 14, 1994