

## Seasonal diel variations of picoplankton and nanoplankton in a subtropical western Pacific coastal ecosystem

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### Abstract

We analyzed seasonal and diel fluctuation patterns of heterotrophic bacteria, *Synechococcus* spp., and nanoflagellates at a coastal station at the southern edge of the East China Sea. *Synechococcus* spp. and nanoflagellates exhibited diel fluctuation at water temperatures above 25°C. Cell concentrations of *Synechococcus* spp. were significantly higher during the evening, whereas those of nanoflagellates were higher during the day. The day and night amounts of heterotrophic bacteria did not differ significantly, and we did not observe diel rhythms in these organisms below 25°C. The fractionation experiments we performed between August and October showed that growth rates of bacteria were high (0.73–1.00  $\mu\text{g C L}^{-1} \text{h}^{-1}$ ) during the day. However, because there was an increase in nanoflagellate grazing, there was no change in the abundance of bacteria over the day. *Synechococcus* spp. was not actively consumed by nanoflagellates during the day, but its rate of production was exceeded by the rate of grazing by nanoflagellates during the night. This out-of-phase *Synechococcus* spp. growth and mortality caused by grazing created diel variations in its abundance. We also found that picoplankton contributed 24–36  $\mu\text{g C L}^{-1} \text{d}^{-1}$  to the microbial loop, and *Synechococcus* spp. and bacteria contributed equally to this carbon flux.

Although studies of the microbial food web (Pomeroy 1974; Azam et al. 1983) have greatly expanded our knowledge of the plankton community and the dynamic interaction between organisms, we have not measured the relative importance of the mechanisms that maintain the ambient levels of population abundance. Pico- and nanoplankton growth and abundance can be affected by several biotic and abiotic factors, including dissolved organic matter, temperature, light, and predation (Burney et al. 1982; Armbrust et al. 1989; Felip et al. 1996). However, the most critical of these factors differs by location and time (Shiah and Ducklow 1994; Goosen et al. 1997).

Although seasonal and interannual changes in abundance of plankton have been studied extensively in various marine environments, a full understanding of the factors regulating long-term variability will require a meaningful understanding of diel variability in microbial activities. The least var-

iable component of plankton, bacteria, does not change much in density and biomass, and bacterial numbers vary by less than one order of magnitude over the course of a year (Cole and Caraco 1993). Despite high physiological rates, the short-term fluctuations of bacterial abundance appear quite stable over a period of a day. Therefore, bacterial production and mortality are usually assumed to be balanced (Simek et al. 2001). However, daily peaks of bacterial production have been reported to occur in the early morning or at midday, a diel rhythm apparently governed by phytoplankton activity (Psenner and Sommaruga 1992; Kuipers et al. 2000) or fluctuation in dissolved carbohydrates (Burney et al. 1982). Irregular peaks in production over a day have also been reported (Gasol et al. 1998). Bacterial mortality has been found to be significantly higher at certain periods of a day (Wikner et al. 1986; Weisse 1999), possibly because of light intensity-induced changes in the feeding rate of protozoa (Ochs 1997; Chen and Chang 1999) or bacteria size during nanoflagellate feeding (González et al. 1990). If diel fluctuations in the abundance of bacterioplankton are to be understood, we need to make clear the degree of synchrony between these processes.

Seasonal variation in the abundance of *Synechococcus* spp., in contrast, has been reported to occur in most tropical

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### Acknowledgments

We thank A. Taniguchi of Tohoku University for his help in establishing our nanoflagellate identification and enumeration techniques. This study was supported by a grant from the National Science Council, Republic of China, NSC 91-2313-B-019-031.

and subtropical oceans throughout the year, with a density ranging from  $10^3$  to  $10^5$  cells  $\text{ml}^{-1}$ , and this variation in abundance has been strongly correlated with the annual cycle of water temperatures (Waterbury et al. 1986). Although the variations of *Synechococcus* abundance depends primarily on production, cell death caused by grazing could be important as well. Data on the short-term variability of *Synechococcus* spp. has shown it to divide about once every 24 h, although mean concentrations vary little from day to day (Partensky et al. 1999). Light is clearly one of the most important factors in regulating the cell cycle (Armbrust et al. 1989), with cell division occurring from late afternoon to early evening (Binder and DuRand 2002). Seasonal and diel variations of *Synechococcus* spp. have been routinely observed in marine picoplankton, but this variation is not always obvious in bacteria. To date, the mechanism behind these variations remains unclear.

The East China Sea is a major coastal ecosystem in the western Pacific. There, picoplankton, *Synechococcus* spp., and heterotrophic bacteria are found to be dominant members. In this region, *Synechococcus* spp. has been reported by Chiang et al. (2002) to have a two-phase seasonal cycle (cold and warm seasons), which suggests that the seasonal variation is controlled by temperature. Substrate supply and temperature are the two most important factors affecting the spatial and seasonal patterns of bacterial rate parameters for heterotrophic bacteria (Shiah et al. 2003). Another important factor could be nanoflagellates, a major predator of picoplankton in the East China Sea, although we lack data on the abundance of nanoflagellates and the rate at which they ingest picoplankton.

Because abundance of picoplankton is controlled by its gain and loss, it is essential to find out the growth and grazing rates of nanoflagellates on *Synechococcus* spp. and heterotrophic bacteria if we are to understand the mechanisms behind diel variation. In this report, diel variations of *Synechococcus* spp., heterotrophic bacteria, and nanoflagellates were measured at a specific location on the southern edge of the East China Sea. Abundance was measured by counting the number of cells; growth and grazing rates were measured by size fractionation. We found clear evidence of diel cycles for *Synechococcus* spp. and nanoflagellates but not for bacteria between June and October. The amplitude of these diel cycles were found to greatly contribute to the degree of synchronization between growth and grazing, as well as to the selective feeding of nanoflagellates.

## Materials and methods

**Sampling**—Samples were collected from July 1999 to September 2001 at a coastal station ( $25^{\circ}9.4'N$ ,  $121^{\circ}46.3'E$ ) that was established on a rocky shore on the northeast coast of Taiwan (Fig. 1). Two 48-h diel variation studies of plankton abundance were conducted from 8 July to 10 July 1999 and from 12 March to 14 March 2000. During each sampling period, surface water was collected every 2 h. Samples for the study of seasonal patterns of diel variation were collected biweekly from June to September and weekly from November to May. On each sampling day, seawater was collected

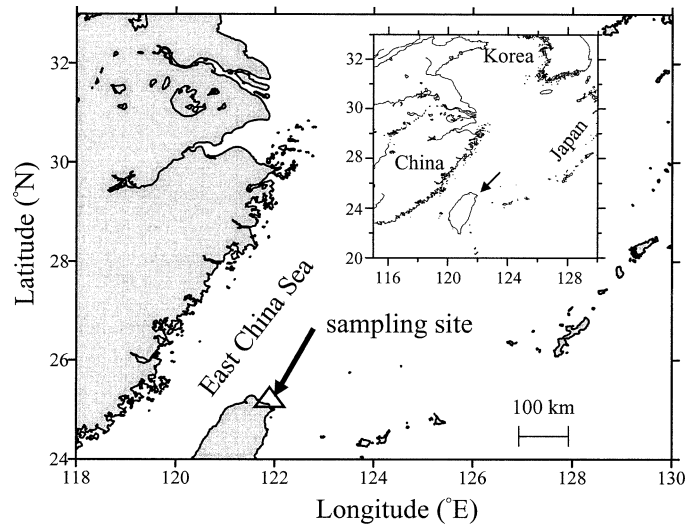


Fig. 1. Study site location.

twice, once between 0900 and 1000 h in the morning and the other between 2100 and 2200 h in the evening (local time). Water temperature was measured immediately after the bucket was cast. All samples were brought to the laboratory within 30 min.

**Plankton abundance**—Samples for the enumeration of pico- and nanoplankton were fixed immediately by adding glutaraldehyde to a final concentration of 1% (v/v). With the use of a Millipore filter with a pore size of  $0.45 \mu\text{m}$  as a pad to obtain a uniform distribution of cells and low pressure ( $<100 \text{ mm Hg}$ ), we filtered the subsamples to be used to measure picoplankton (2 ml) and nanoflagellates (20 ml) onto 0.2- and  $0.8\text{-}\mu\text{m}$  pore size black Nuclepore filters, respectively. The cells remaining on the filter membranes were stained with 4',6-diamidino-2-phenylindole at a final concentration of  $1 \mu\text{g ml}^{-1}$  (Porter and Feig 1980) and were examined by epifluorescence microscopy ( $\times 1,000$ ; Nikon Optiphot-2). Bacteria and nonpigmented nanoflagellates were identified by their blue fluorescence under ultraviolet illumination. Autotrophic picoplankton (cyanobacteria *Synechococcus* spp.) in 4–10 ml of seawater were collected on a  $0.2\text{-}\mu\text{m}$  pore size Nuclepore filter without staining. Cyanobacteria and pigmented nanoflagellates were identified by their orange and red autofluorescence under blue excitation light. To obtain reliable estimates of abundance, we counted the cells of bacteria, *Synechococcus* spp., and nanoflagellates with 10, 30, and 50 fields of view, respectively.

**Time lag correlation analysis**—On the basis of data collected during the 48-h sampling periods, a series of product-moment correlation coefficients were calculated for *Synechococcus* abundance at time  $t_i$  and nanoflagellate abundance at time  $(t_i + \Delta t)$ , in which the time lag  $\Delta t$  varied from 0 to 24 h. The time lag that generated the highest correlation coefficient was used as an estimate of the duration between peak abundances of *Synechococcus* and nanoflagellates, provided that the coefficient was significantly different from zero.

**Growth and grazing rates**—By differential method (Wright and Coffin 1984), we estimated growth and grazing rates three times from August to October 2002. On the day of each experiment, seawater was collected separately between 0900 and 1000 h and at nighttime between 2100 and 2200 h. It was immediately transported to the laboratory. Samples were treated twice to remove predators of different sizes. A 2- $\mu\text{m}$  pore size polycarbonate filter was used to remove predators of bacteria and *Synechococcus* spp., and a 10- $\mu\text{m}$  pore size polycarbonate filter was used to remove predators of nanoflagellates. Each size fraction was then transferred into polycarbonate bottles to a volume of 125 ml (run in triplicate). The bottles were incubated in a water bath at in situ temperature and under continuous illumination at  $\sim 150 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  for 6 h for samples collected in the morning and in darkness for the same length of time for samples collected in the evening. At the beginning and end of each incubation period, triplicate samples (30 ml) were taken to count pico- and nanoplankton as described above.

Growth rates ( $\mu$ ,  $\text{h}^{-1}$ ) of bacteria and *Synechococcus* spp. were calculated on the basis of the results from the  $<2\text{-}\mu\text{m}$  filtrates, and those of nanoflagellates were calculated from the  $<10\text{-}\mu\text{m}$  filtrates according to the equation

$$\mu = (\ln N_f - \ln N_i)/(T_f - T_i)$$

where  $N_i$  and  $N_f$  are cell concentrations ( $\text{cells ml}^{-1}$ ) at the beginning ( $T_i$ ) and end ( $T_f$ ) of the incubation period in corresponding size fractions.

Microbial abundance was converted into carbon biomass ( $B$ ,  $\mu\text{g C L}^{-1}$ ) with the equation

$$B = N \times C$$

where  $N$  is cell density ( $\text{cells ml}^{-1}$ ) and  $C$  is estimated cell carbon content ( $\text{fg C cell}^{-1}$ ). Estimated cell carbon used was 15  $\text{fg C cell}^{-1}$  for bacteria (Caron et al. 1995), 250  $\text{fg C cell}^{-1}$  for picophytoplankton, and 220  $\text{fg C } \mu\text{m}^3$  for nanoflagellates (Børsheim and Bratbak 1987). For cell volume of nanoflagellates, linear dimensions (length and width) of at least 20 cells were measured in each sample, and the cell volume was calculated as an elliptical sphere.

Production rates ( $P$ ,  $\mu\text{g C L}^{-1} \text{ h}^{-1}$ ) of bacteria and cyanobacteria were estimated from the  $<2\text{-}\mu\text{m}$  filtrates with the equation

$$P = \mu \times B_i$$

where  $B_i$  is the in situ cell biomass ( $\mu\text{g C L}^{-1}$ ) at the sampling time. Production rates ( $\mu\text{g C L}^{-1} \text{ h}^{-1}$ ) of nanoflagellates were similarly estimated in the  $<10\text{-}\mu\text{m}$  filtrates.

Consumption rates of nanoflagellates ( $G$ ,  $\mu\text{g C L}^{-1} \text{ h}^{-1}$ ) on picoplankton were calculated according to the equation

$$G = (P_{\text{pico}})_{2\mu\text{m}} - (P_{\text{pico}})_{10\mu\text{m}}$$

where  $P_{2\mu\text{m}}$  and  $P_{10\mu\text{m}}$  are the production rates ( $\mu\text{g C L}^{-1} \text{ h}^{-1}$ ) of picoplankton (bacteria and *Synechococcus* spp.) in the  $<2\text{-}\mu\text{m}$  and the  $<10\text{-}\mu\text{m}$  filtrates.

## Results

**Environmental conditions**—The seasonal dynamics of temperature and nitrate during the study period are given in

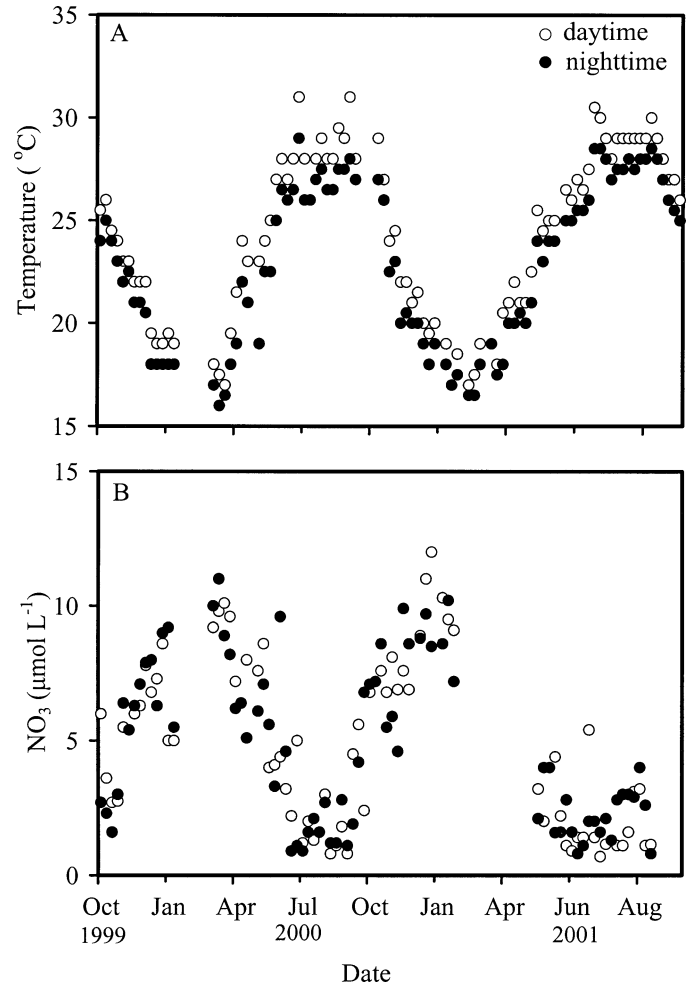


Fig. 2. (A) Surface water temperatures and (B) nitrate concentrations measured at the coastal station from October 1999 to September 2001.

Fig. 2A,B. Surface water temperatures were around 16°C in March and increased gradually to 29°C in June. They stabilized from June to September and then decreased thereafter. The water temperature was constantly above 25°C from June to October. Daytime temperatures were generally 0.5–1.5°C higher than nighttime temperatures (Fig. 2A), and the seasonal course of temperatures was similar for both years. Salinity ranged from 33.1 to 34.3 over a year. A drop to a salinity below 34 was probably caused by rainfall.

Average nitrate concentration was high between November and May, when it reached 12  $\mu\text{mol L}^{-1}$ . From June to October, average nitrate concentration decreased to about 1  $\mu\text{mol L}^{-1}$ . Both day and night nitrate concentrations were negatively correlated with water temperature ( $\text{NO}_3 = -0.65\text{Temp} + 20.7$ ,  $r^2 = 0.77$ , day;  $\text{NO}_3 = -0.65\text{Temp} + 19.8$ ,  $r^2 = 0.74$ , night; Fig. 2B). Nitrate concentrations at day and night did not differ significantly, and they changed in a similar pattern to that found for daytime concentrations.

**Diel characteristics of picoplankton and nanoflagellates**—During the 48-h sampling period taken in July 1999, abundance of nanoflagellates and *Synechococcus* spp.

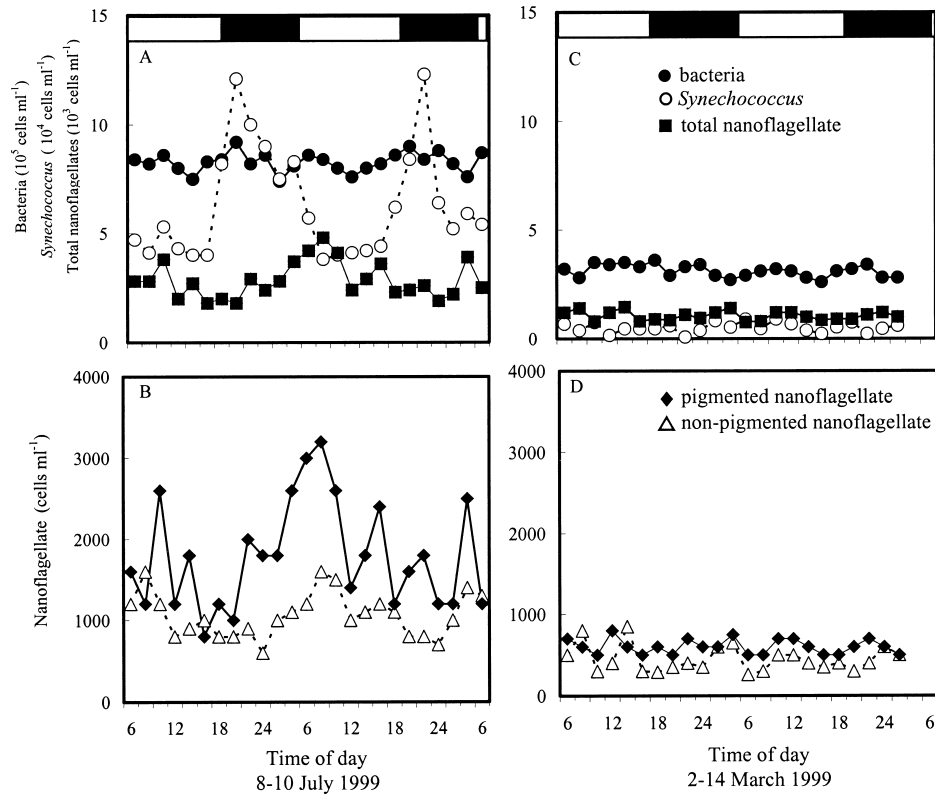


Fig. 3. Changes in picoplankton abundance at different times of the day between 8 and 10 July 1999 (A,B) and between 12 and 14 March 2000 (C,D). Upper panels (A,C) depict changes in heterotrophic bacteria, *Synechococcus*, and total nanoflagellates abundances; lower panels (B,D) depict changes in pigmented and nonpigmented nanoflagellate abundances.

showed a diel variation pattern (Fig. 3A). *Synechococcus* spp. abundance ranged from  $4 \times 10^4$  to  $12.4 \times 10^4$  cells  $ml^{-1}$ , with high values recorded in the early evening samples. Concentrations of nanoflagellates were low during the period of high *Synechococcus* spp. abundance, with nanoflagellates ( $\sim 5 \times 10^3$  cells  $ml^{-1}$ ) peaking in the early morning (Fig. 3A). Numbers of pigmented nanoflagellates varied between  $0.9$  and  $3.1 \times 10^3$  cells  $ml^{-1}$ . Nonpigmented nanoflagellates varied similarly, with a somewhat smaller amplitude (Fig. 3B). Correlation analysis was performed between these two organisms with different time lags. The nanoflagellate peak lagged about 14 h behind the *Synechococcus* spp. peak (Fig. 4); the best correlation was found between *Synechococcus* and total nanoflagellates rather than its components. However, this diel variation in *Synechococcus* spp. and nanoflagellates was not observed during March 2000. On the sampling day in March 2000, *Synechococcus* spp. abundance ranged from  $0.2$  to  $0.8 \times 10^4$  cells  $ml^{-1}$ , and total nanoflagellates ranged from  $0.7$  to  $1.4 \times 10^3$  cells  $ml^{-1}$  (Fig. 3C). A comparison between these 24-h sampling dates showed that the daily mean abundances in March were much lower than those in July. Abundance for bacteria ranged from  $7.6$  to  $8.8 \times 10^5$  cells  $ml^{-1}$  during July 1999 (Fig. 3A) and did not show a clear pattern of diel variation. On sampling days in March, the number of pigmented and nonpigmented nanoflagellates were also low (Fig. 3D).

*Seasonal patterns of diel variations of picoplankton*—Our 2-yr comparison indicated no significant difference between daytime and nighttime bacteria abundance (Figs. 5A, 6A). Bacteria exhibited a clear annual cycle with high values ( $\sim 1.0$ – $1.5 \times 10^6$  cells  $ml^{-1}$ ) occurring between June and September, and low values ( $\sim 0.2 \times 10^6$  cells  $ml^{-1}$ ) in March. This pattern of seasonal variation corresponded well with the annual cycle of temperatures (Fig. 2A).

The seasonal trend of *Synechococcus* spp. was similar to that of bacteria. Concentrations of *Synechococcus* spp. were low ( $2$ – $5 \times 10^3$  cells  $ml^{-1}$ ) during periods of low temperature (January to March; Fig. 5B). When temperatures rose above  $25^\circ C$  at the beginning of June, abundance increased to  $1 \times 10^4$  cells  $ml^{-1}$  and remained relatively high until October. Between June and September, nighttime *Synechococcus* spp. concentrations were constantly higher than daytime concentrations, indicating the presence of a diel cycle (paired *t*-test,  $p < 0.05$ ; Fig. 6B). Also, nondividing *Synechococcus* spp. cells increased sharply during the daily dark period (Fig. 7). However, during October–May (water temperature  $< 25^\circ C$ ), no significant diel variation in *Synechococcus* spp. was detected (Fig. 6B).

*Seasonal patterns of diel variations of nanoflagellates*—Nanoflagellate abundance ranged from  $2 \times 10^2$  to  $6 \times 10^3$  cells  $ml^{-1}$  over the year (Fig. 5C). Similar to bacteria and

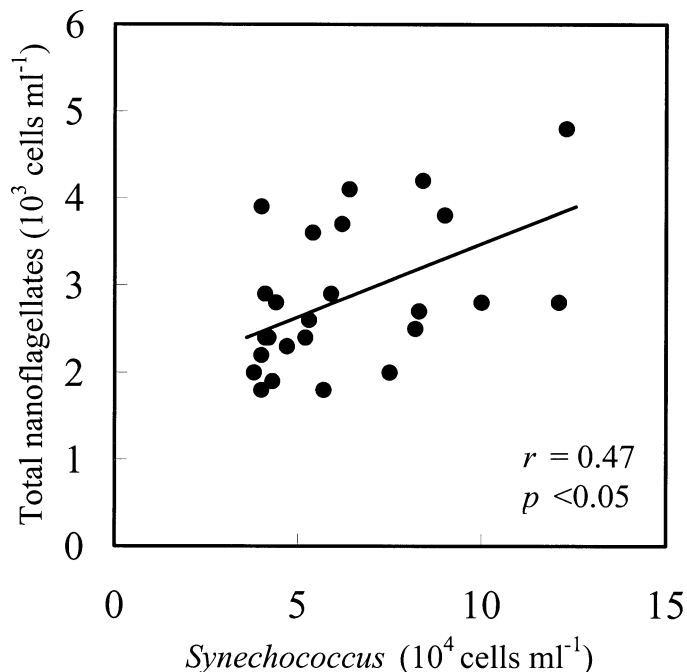


Fig. 4. Correlation between *Synechococcus* and total nanoflagellate abundances measured between 8 and 10 July 1999. A time lag of 14 h was used when comparing nanoflagellate abundances. The solid line marks the position of the principle axis of equal-frequency ellipses.

*Synechococcus* spp., nanoflagellate abundance exhibited a clear seasonal cycle, with high values during the period from June to October and low values in other months. The seasonal patterns were similar over both years of investigation. Differences in daytime and nighttime abundance were clearly seen between June and October. In contrast to the diel variation of *Synechococcus* spp., nanoflagellate abundances were generally higher in the daytime during this period (Fig. 5C). No diel variation was detected in nanoflagellate abundance between November and May. Pigmented nanoflagellates consistently accounted for ~60–75% of the total flagellate population year-round. Both diel and seasonal variations of pigmented nanoflagellates were similar to those of total nanoflagellates. (Fig. 5C,D,E).

**Production rates between August and October**—Because diel variations in abundances were most obvious during the period of water temperature  $>25^{\circ}\text{C}$ , we performed three size fractionation experiments in August, September, and October 2003 to investigate how growth and grazing rates determined the differences between day and night. Production of bacteria varied between 0.73 and 1.00  $\mu\text{g C L}^{-1} \text{h}^{-1}$  in the daytime (mean 0.89  $\mu\text{g C L}^{-1} \text{h}^{-1}$ ; Table 1). All nighttime values were lower and ranged between 0.13 and 0.61  $\mu\text{g C L}^{-1} \text{h}^{-1}$  (mean 0.31  $\mu\text{g C L}^{-1} \text{h}^{-1}$ ; Table 1). Daytime production was about threefold higher than nighttime production.

In contrast, *Synechococcus* spp. were found to have a higher production rate at night, ranging from 0.50 to 1.38  $\mu\text{g C L}^{-1} \text{h}^{-1}$  (mean 0.84  $\mu\text{g C L}^{-1} \text{h}^{-1}$ ; Table 1). The day-

time production of *Synechococcus* spp. was lower, ranging from 0.21 to 0.50  $\mu\text{g C L}^{-1} \text{h}^{-1}$ . The production rates of nanoflagellates ranged from 0.50 to 0.57  $\mu\text{g C L}^{-1} \text{h}^{-1}$  during the daytime and from 0.30 to 0.48  $\mu\text{g C L}^{-1} \text{h}^{-1}$  during the night (Table 1). No significant differences were found ( $t$ -test,  $p > 0.05$ ) between daytime and nighttime nanoflagellate production rates.

**Grazing rates between August and October**—Size fractionation studies revealed that nanoflagellates ingested more bacterial carbon ( $\sim 0.70$ – $0.90 \mu\text{g C L}^{-1} \text{h}^{-1}$ ; Table 1) during the daytime, but they only ingested  $\sim 0.13$ – $0.30 \mu\text{g C L}^{-1} \text{h}^{-1}$  (Table 1) at night. The mean values for daytime and nighttime were 0.82 and 0.22  $\mu\text{g C L}^{-1} \text{h}^{-1}$ , representing a fourfold increase during the day. This finding showed that nanoflagellates consumed nearly all the bacteria that was produced during the daytime and nighttime.

A different pattern was found for the grazing rates of nanoflagellates on *Synechococcus* spp. Grazing mortality of *Synechococcus* spp. remained low ( $\sim 0.10$ – $0.20 \mu\text{g C L}^{-1} \text{h}^{-1}$ ; Table 1) during daytime but became higher during the nighttime ( $\sim 0.55$ – $1.50 \mu\text{g C L}^{-1} \text{h}^{-1}$ ), with a mean of 0.97  $\mu\text{g C L}^{-1} \text{h}^{-1}$  (Table 1). All nighttime *Synechococcus* spp. grazing rates were higher than nighttime production rates.

**Diel variation in carbon fluxes between August and October**—Regardless of time of day, except for September nighttime values (38%), nanoflagellates removed 87–150% of the bacteria that had been produced (Table 1). Mean grazing-to-production ratio of daytime and nighttime was 95% and 96%, respectively. There was a strong equilibrium between diel production and grazing, which approximated that found for bacteria.

Nanoflagellates consumed only 26–53% of *Synechococcus* production in the daytime, with a mean of 42% (Table 1), meaning that about 60% of *Synechococcus* spp. production was not consumed in the daytime. However, with the marked nighttime increase of *Synechococcus* spp. abundance came an increase in nanoflagellate grazing pressure on *Synechococcus* spp. (Table 1). Grazing pressure on *Synechococcus* spp. exceeded its production at night (Table 1).

## Discussion

**Seasonal changes in the abundance of picoplankton and nanoplankton**—Seasonal variation of bacterial abundance at the study site ranged from 0.2 to  $1.5 \times 10^6$  cells  $\text{ml}^{-1}$ , with high values occurring from June to September followed by a marked decrease in October (Fig. 5A). We found seasonal variability in bacterial abundance to be positively influenced by temperature (Fig. 8A), which is not surprising because bacterial abundance and production in aquatic ecosystems have been shown to vary with temperature (Hoch and Kirchman 1993). The importance of temperature as a positive regulator of marine bacterial growth rate is well recognized (White et al. 1991). However, in temperatures ranging between  $25^{\circ}\text{C}$  and  $29^{\circ}\text{C}$ , bacterial abundance did not increase with temperature (Fig. 8A), suggesting that there might be another mechanism behind the control of bacterial activity. Besides being influenced by temperature, population dynam-

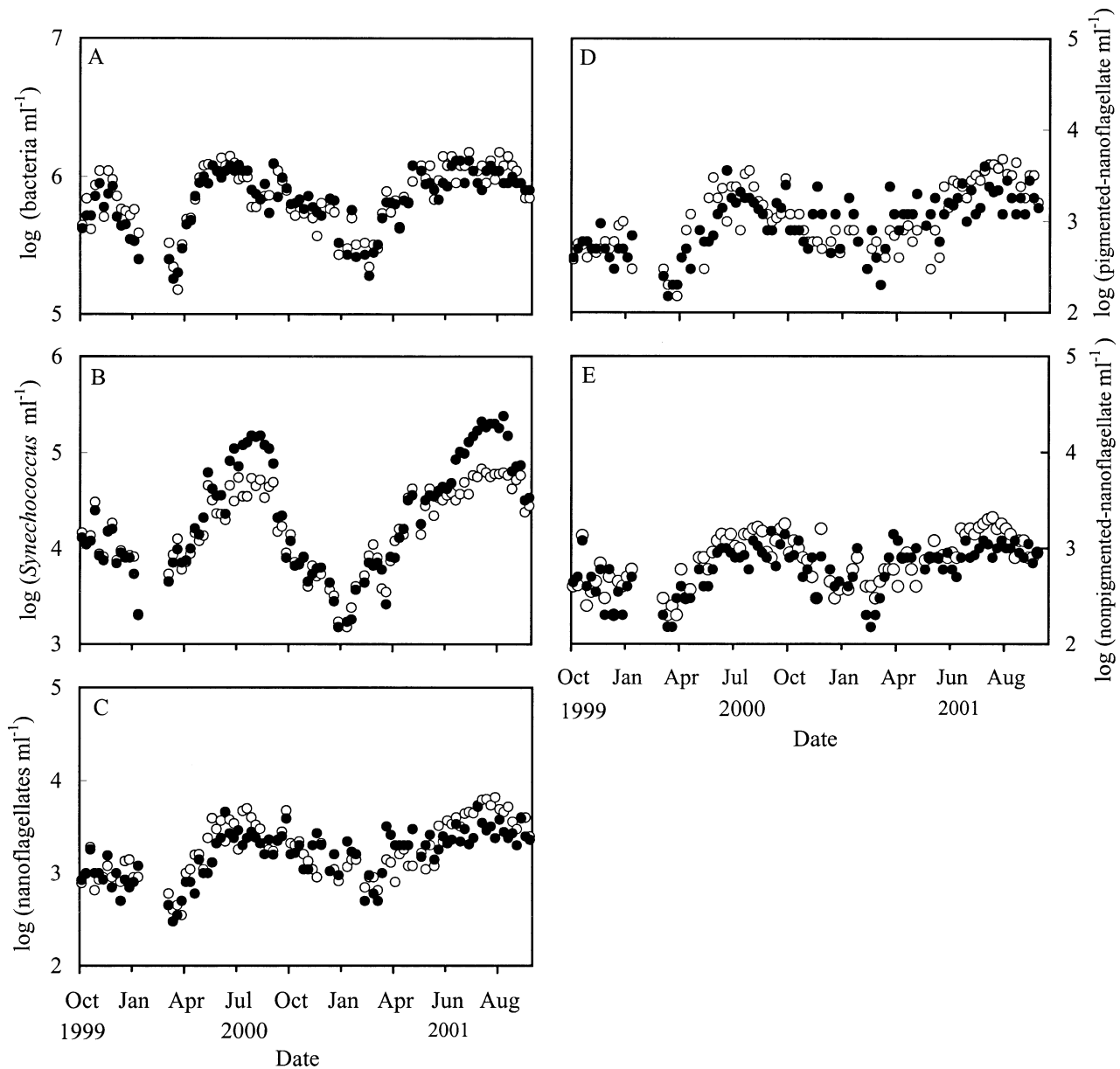


Fig. 5. Comparisons between day and night abundances in pico- and nanoplankton over the 2-yr investigation period. (A) Bacteria, (B) *Synechococcus* spp., (C) total nanoflagellates, (D) pigmented nanoflagellate, and (E) nonpigmented nanoflagellate.

ics in the microbial food web is also influenced by resource availability and predator activity. Top-down controls such as grazing pressure are thought to set limits on bacterial abundance (Boissonneault-Cellineri et al. 2001). Our size fractionation experiments showed that nanoflagellates consumed nearly all the bacterial production between August and October (Table 1). The strong grazing activity clearly prevented increases in bacterial abundance at our study site at temperatures  $>25^{\circ}\text{C}$ .

The seasonal trends of *Synechococcus* spp. abundance closely followed the annual cycle of temperatures (Fig. 5B). Linear relationships could be established between *Synechococcus* spp. abundance and temperature ( $r^2 = 0.81$ , night;  $r^2 = 0.77$ , day). This finding is similar to the results of Chang et al. (1996), who suggested that *Synechococcus* spp. abun-

dance in a subtropical western Pacific coastal ecosystem was closely related to water temperature.

The number of nanoflagellates in aquatic environments are typically positively correlated with number of bacteria, as reported in one broad-scale investigation (Sanders et al. 1992). The strong correlation between number of bacteria and nanoflagellates indicates that seasonal fluctuations of nanoflagellates occur in response to fluctuations in abundance of their prey. The results of this study show a positive correlation between log-transformed abundances of bacteria and that of nanoflagellates. A similar relationship was found between *Synechococcus* spp. and nanoflagellates over the year (Fig. 9A,C).

At our study site, both pigmented and nonpigmented nanoflagellates seemed to participate in the grazing of bacteria

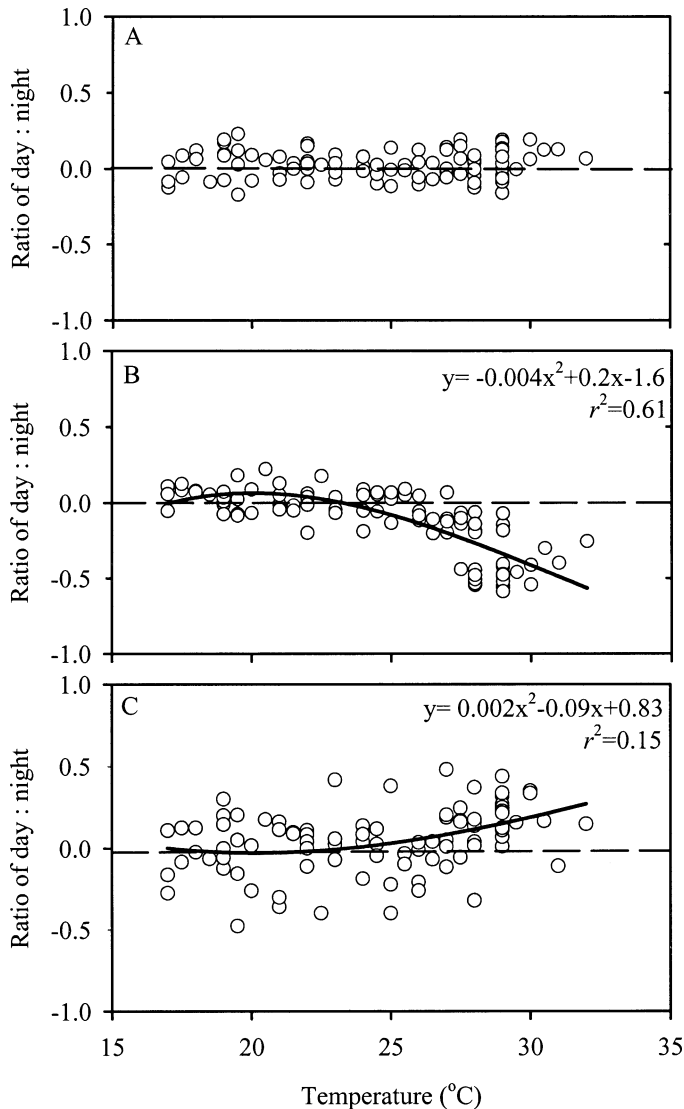


Fig. 6. Ratio between day and night (log-transformed) bacteria (A), *Synechococcus* spp. (B), and total nanoflagellate (C) abundances as a function of temperature during the investigation period. Curves were regression lines obtained by quadratic equation curve fitting.

and *Synechococcus*. The fraction occupied by the pigmented cells was quite stable over the year, and both pigmented and nonpigmented nanoflagellates behaved similarly in a diel cycle (Fig. 5D,E). These findings suggest that they occupy similar niches in the microbial loop. In addition, assuming that only nonpigmented nanoflagellates were capable of consuming picoplankton, we did not observe a tighter correlation between nonpigmented nanoflagellates and bacteria/*Synechococcus* (Fig. 9B,D). Our results are in agreement with recent evidence that phagotrophy was used by both pigmented and nonpigmented nanoflagellates to obtain their carbon source, and these two types of nanoflagellates possess similar grazing rates (Hall et al. 1993).

*Causes of diel variations in picoplankton abundance*—Fractionation was a reliable method of estimating growth

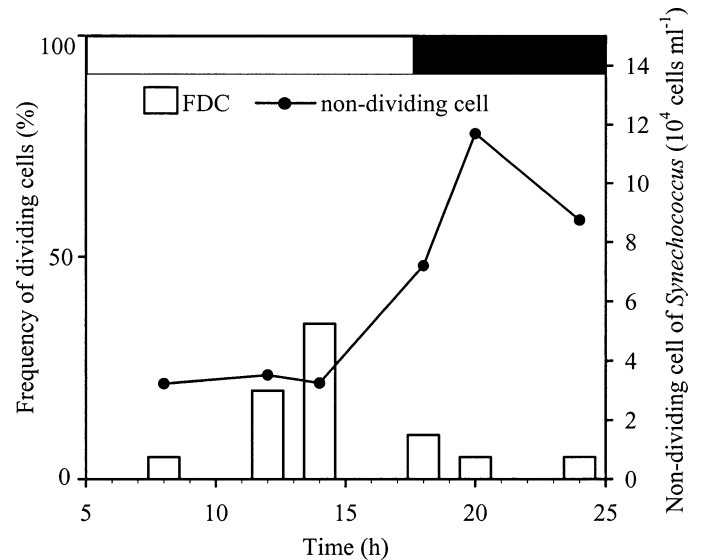


Fig. 7. Diel dynamics of nondividing cells and the frequency of dividing cells of *Synechococcus* spp. collected between 8 and 10 July 1999. Dark bar denotes nighttime hours.

and grazing rates. The 2- $\mu$ m pore filter was very effective in removing grazers of bacteria and *Synechococcus* because no nanoflagellate could be found in the filtrate when at least 50 fields of view were examined under a microscope. With regard to the influences of fractionation on *Synechococcus*, from 3% to 9% of cells were retained on the 2- $\mu$ m filter membrane. Our measured *Synechococcus* growth rates indicated that an 18–45% increase in cell number occurred during the 6-h incubation time, suggesting that cell populations still proliferated actively.

While doing our fractionation experiments from August to October, we found that both the production of bacteria and mortality of bacteria were higher during the daytime (Table 1). Although bacterial production was found to have significant diel variations, bacterial abundance did not (Fig. 6), a finding similar to those of other studies on the stability of the bacterial community (Torreton and Dufour 1996; Jugnia et al. 2000). Nanoflagellate preference for grazing on larger bacteria (Chrzanowski and Šimek 1990) might contribute to observed patterns. In our study, nanoflagellates were found to graze on large and actively dividing cells during the daytime at a rate (Table 1) capable of reducing the surplus of bacteria and balancing it out. Because the grazing losses varied at the same time as the growth rates (Table 1), bacterivory appears to be the main factor involved in controlling bacterial abundance during the warmer part of a year.

*Synechococcus* spp. in marine environments is known to have higher division rates at dusk (Jacquet et al. 1998; Vaulot and Marie 1999). Accompanying this process are decreases in mean cell size of *Synechococcus* spp. population and increases in cell abundance and fraction of nondividing cells (Fig. 7). However, because the diel rhythm in bacterial production did not create rhythms in bacterial abundance (Fig. 6), the observed diel variation in *Synechococcus* spp. abundance probably had a different control mechanism. Our experimental data showed a clear diel variation in the

Table 1. Diel variations of production rate, grazing rate, and the grazing/production (G:P) ratio of picoplankton, as well as the production and gross growth efficiency (GGE) of nanoflagellates between August and October 2002. The values given in parentheses denote the SD. Daytime values were obtained from incubations at 1000 to 1600 h and nighttime values were obtained from incubations at 2200 to 0400 h.

| Month | Bacteria       |                |                |                |             |            | Synechococcus  |                |                |                |            |             | Nanoflagellates |                |              |
|-------|----------------|----------------|----------------|----------------|-------------|------------|----------------|----------------|----------------|----------------|------------|-------------|-----------------|----------------|--------------|
|       | Production     |                | Grazing        |                | G:P (%)     |            | Production     |                | Grazing        |                | G:P (%)    |             | Production      |                | GGE (%)      |
|       | Day            | Night          | Day            | Night          | Day         | Night      | Day            | Night          | Day            | Night          | Day        | Night       | Day             | Night          |              |
| Aug   | 1.00<br>(0.26) | 0.13<br>(0.05) | 0.87<br>(0.24) | 0.13<br>(0.04) | 87<br>(21)  | 100<br>(6) | 0.50<br>(0.15) | 1.38<br>(0.33) | 0.13<br>(0.04) | 1.50<br>(0.28) | 26<br>(12) | 109<br>(20) | 0.57<br>(0.12)  | 0.48<br>(0.16) | 43.0<br>(14) |
| Sep   | 0.73<br>(0.31) | 0.61<br>(0.10) | 0.70<br>(0.24) | 0.23<br>(0.08) | 100<br>(26) | 38<br>(7)  | 0.21<br>(0.09) | 0.63<br>(0.12) | 0.10<br>(0.03) | 0.91<br>(0.31) | 48<br>(18) | 144<br>(16) | 0.55<br>(0.10)  | 0.36<br>(0.08) | 50.5<br>(11) |
| Oct   | 0.93<br>(0.27) | 0.20<br>(0.06) | 0.90<br>(0.19) | 0.30<br>(0.06) | 97<br>(21)  | 150<br>(6) | 0.38<br>(0.06) | 0.50<br>(0.09) | 0.20<br>(0.06) | 0.55<br>(0.22) | 53<br>(8)  | 110<br>(22) | 0.50<br>(0.09)  | 0.30<br>(0.11) | 40.0<br>(10) |
| Mean  | 0.89           | 0.31           | 0.82           | 0.22           | 95          | 96         | 0.36           | 0.84           | 0.14           | 0.97           | 42         | 121         | 0.54            | 0.38           | 44.5         |

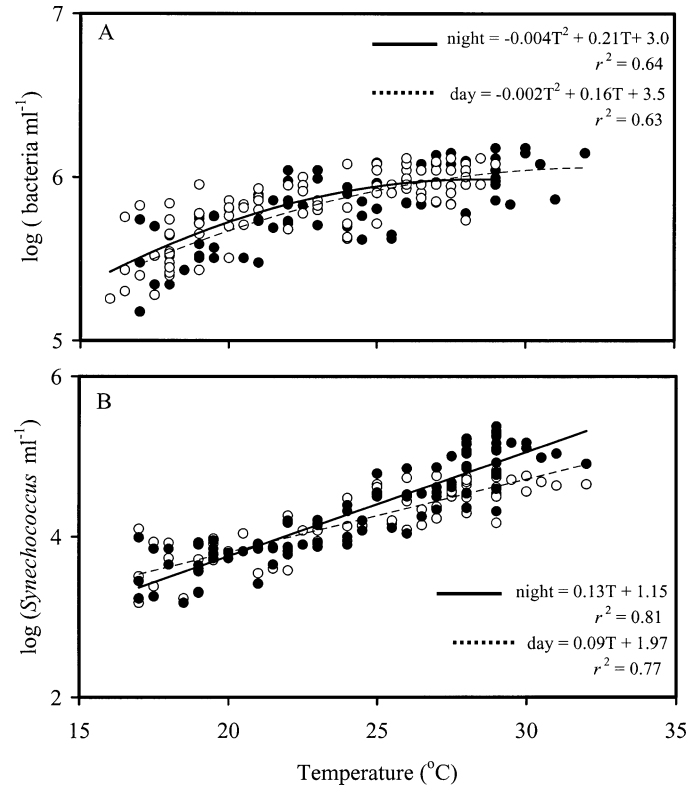


Fig. 8. Relationships between picoplankton abundance and temperatures. (A) Bacteria versus temperature. Curves were regression lines obtained by quadratic equation curve fitting. (B) *Synechococcus* spp. versus temperature. Curves were regression lines obtained by linear equation curve fitting.

amount of *Synechococcus* spp. consumed by nanoflagellates with the highest feeding rates at night, a finding similar to that of Dolan and Šimek (1998). As a result, *Synechococcus* spp. production exceeded its consumption by grazers in the daytime, and the population of *Synechococcus* began to rebuild by cell division throughout this period.

Patchy distribution plus tidal movement can also cause rhythmic variation in picoplankton abundance. This was unlikely at our study site for several seasons. On sampling days with conspicuous diel variation, *Synechococcus* abundance was constantly higher at night, but nanoflagellate abundance was constantly higher in the daytime. This pattern did not match the tidal rhythm. In addition, the semidiurnal tides at our study site would have created more than one peak per day. However, experiments to evaluate tidal influence on *Synechococcus* diel variation will be a meaningful research direction in the future.

Prey size seems to be a reasonable explanation for the increased consumption of bacteria by nanoflagellates in the daytime but of *Synechococcus* spp. at night. Benni (1994) reported that the size ratio between predators and their optimal prey is 3:1 for nanoflagellates. If this is the case, nanoflagellates of 3–5 μm, which dominated the nanoflagellate community at our study site, should prefer prey of 1–1.5 μm. Because actively dividing *Synechococcus* spp. cells were about 2 μm, they were too large for nanoflagellates to feed on in the daytime. Nanoflagellate feeding rates were

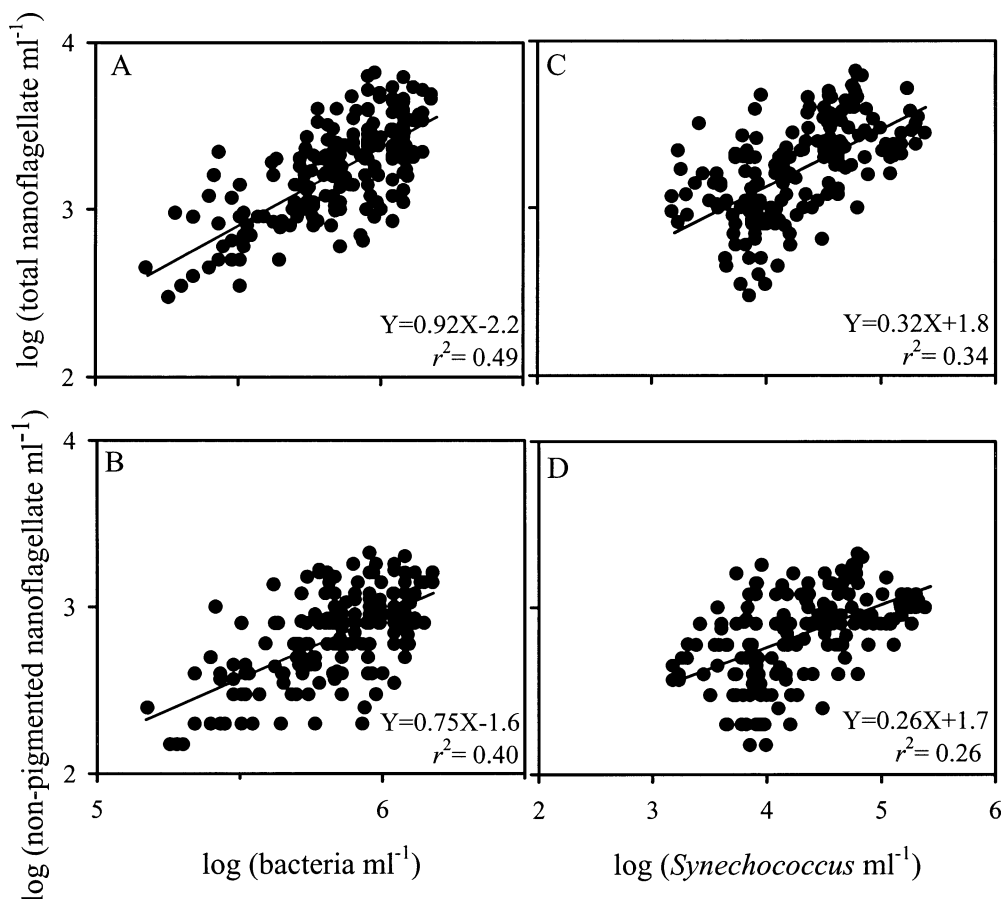


Fig. 9. Relationships between (A) bacterial abundance and total nanoflagellate abundances, (B) bacteria and nonpigmented nanoflagellate abundance, (C) *Synechococcus* spp. and total nanoflagellate abundances, and (D) *Synechococcus* spp. and nonpigmented nanoflagellates during the study period. Solid lines were generated by linear regression.

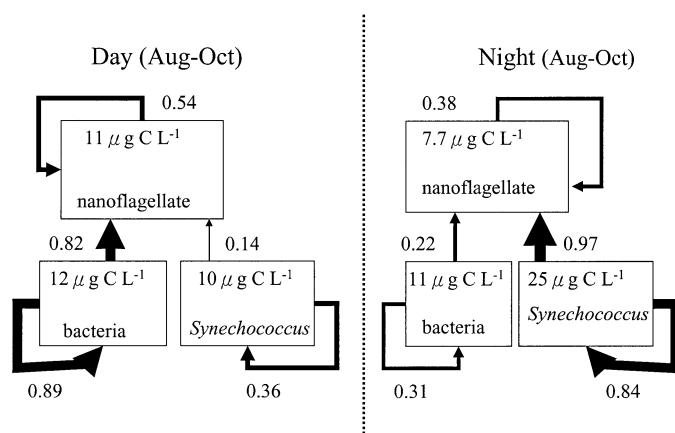


Fig. 10. Schematic carbon flow diagrams depicting diel variations in energy transfer between nanoflagellates and picoplankton in a subtropical Western Pacific coastal ecosystem from August to October. The numbers above individual picoplankton and nanoflagellates refer to biomass. The number next to looped arrows represent production rates ( $\mu\text{g C L}^{-1} \text{ h}^{-1}$ ). Straight arrows pointing to nanoflagellates indicate grazing rates ( $\mu\text{g C L}^{-1} \text{ h}^{-1}$ ).

likely to increase at night after *Synechococcus* cell division had peaked and the cells were smaller (Table 1; Fig. 10), although several recent studies have not found size selection in some of the smallest heterotrophic nanoflagellates (Guilou et al. 1999). Other factors, such as food texture and chemical signals, that could cause selective feeding need to be investigated in the future.

The lower temperatures during the colder parts of a year brought about a rapid decrease in abundance of picoplankton (Fig. 5A,B). Although no fractionation experiment was conducted, a logical deduction is that growth rates of picoplankton were close to zero during this period, resulting in the disappearance of diel rhythms in population growth and lower abundances of bacteria and *Synechococcus* spp. (Fig. 5A,B). Berninger et al. (1991) suggested that the threshold density of bacteria for effective nanoflagellate grazing was near  $3 \times 10^5$  cells  $\text{ml}^{-1}$  and the feeding threshold of picocyanobacteria is  $5 \times 10^4$   $\text{ml}^{-1}$ . From their findings, we can infer that between November and May, nanoflagellates probably faced a shortage of prey, and diel rhythms of picoplankton abundance disappeared.

*Diel variation of picoplankton carbon flux from August to October*—The results of our study clearly indicate that nan-

oflagellates largely depend on bacteria as an energy source in the daytime during the warm season. The average feeding rate was  $0.82 \mu\text{g C L}^{-1} \text{h}^{-1}$ , which is equivalent to an ingestion/production ratio of 95% in the daytime (Fig. 10). Wikner et al. (1990), in a study that reported ingestion/production ratios of bacteria, showed that nanoflagellate predators consumed 40–430% of bacterial production. In our study, nanoflagellates grazed on *Synechococcus* spp. at a mean rate of  $0.14 \mu\text{g C L}^{-1} \text{h}^{-1}$  in the daytime, but at night they consumed *Synechococcus* spp. at a mean rate of  $0.97 \mu\text{g C L}^{-1} \text{h}^{-1}$  (Fig. 10). This value was equivalent to a *Synechococcus* spp. ingestion rate of 1.1 cells flagellate<sup>-1</sup> h<sup>-1</sup>, which is in accordance with the 0.02 and 2.6 cells flagellate<sup>-1</sup> h<sup>-1</sup> rates measured by Kuosa (1991), who also used the size fractionation method.

Both bacteria and *Synechococcus* spp. can be consumed by nanoflagellates and are important energy sources in the microbial loop (Boissonneault-Cellineri et al. 2001; Callieri et al. 2002). On the basis of experiments conducted around noon, Christaki et al. (2001) concluded that picoautotrophic carbon is unlikely to enter the microbial food web through nanoflagellate grazing. In our site measurements, if we only took the daytime measurements into consideration, carbon flux followed a similar pattern (Fig. 10). However, our night measurements greatly changed this view. Because abundance of *Synechococcus* spp. was higher at night, this organism became the major food source for nanoflagellates (Fig. 10). Adding the day and night values together, we found that the daily carbon contribution from *Synechococcus* spp. to the microbial loop was about the same as that from bacteria. This conclusion illustrates the importance of taking multiple measurements over a whole day in an ecosystem with significant diel rhythms.

*Growth efficiency of nanoflagellates*—On the basis of the measured values, we calculated the carbon required for nanoflagellates to sustain the observed production rates. Our calculations, based on the daily mean values of growth efficiency of nanoflagellates during the period from June to October, ranged from 43% to 50.5% (mean 44.5%; Table 1). Our estimates were close to those reported by Jugnia et al. (2000), implying that bacteria and *Synechococcus* spp. were the dominant prey for nanoflagellates during the period from June to October.

Our simultaneous examination of abundances of bacteria, *Synechococcus* spp., and nanoflagellates showed a clear seasonal cycle. When water temperatures exceeded 25°C, both *Synechococcus* spp. and nanoflagellates exhibited diel rhythms in abundance, but bacteria did not. On the basis of our size fractionation measurements, nanoflagellate grazing was in phase with bacterial growth, explaining the lack of diel variation in bacterial abundance. In contrast, with *Synechococcus* spp., nanoflagellate grazing was out of phase at night, 14 h after division of *Synechococcus* spp. cells had peaked, creating a diel rhythm. By including night measurements of *Synechococcus* growth rate and mortality, we found both *Synechococcus* spp. and bacteria to contribute equally as sources of organic carbon to the microbial loop.

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Received: 17 June 2004

Accepted: 23 February 2005

Amended: 8 March 2005