Biomass and distribution of filamentous cyanobacteria, *Trichodesmium* spp., in spring in the East China Sea

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Abstract: *Trichodesmium* biovolume and its distributional pattern were investigated in spring in the East China Sea. It showed surface-biased distribution at all stations where it was detected. The maximum biovolume in each water column was 0–$4.4 \times 10^7 \, \mu m^3 \, L^{-1}$, and it was sometimes larger than that of coccoid cyanobacteria (8.9×10^6–$2.0 \times 10^7 \, \mu m^3 \, L^{-1}$). Horizontal distribution of *Trichodesmium* around the boundary between continental shelf water and Kuroshio water showed different patterns between a large-sized fasciculated form and other forms; the former was more abundant on the Kuroshio side and the latter, small-sized fasciculated form or separate filamentous form, more abundant on the continental shelf side, while the abundance of all types varied temporally. Considering that the large fasciculated form may develop from the separate filamentous form or the small fasciculated form, and it may be more efficient for N_2-fixation under an aerobic conditions, this contrastive horizontal distribution might reflect an adaptive enlargement process in *Trichodesmium* colonies accompanied with passive transport from continental shelf water to more oligotrophic Kuroshio waters.

Key words: biovolume, distribution, East China Sea, *Trichodesmium* spp.

Introduction

Marine filamentous cyanobacteria, *Trichodesmium* spp., generally occur in the tropical and subtropical zones of oligotrophic oceans (e.g. Capone et al. 1997). They have a number of minute gas vesicles occurring throughout the cell, and hence frequently show surface congregation under stratified water conditions, owing to their positive buoyancy (Carpenter 1983a, Capone & Carpenter 1999). Their photosynthetic growth or passive accumulation in the surface layer are sometimes strong enough to form a red tide even in offshore areas. Furthermore, they are recognized as a unique diazotroph: N_2-fixation is carried out in oxygen-saturated surface waters without employing a heterocyst (Bergman et al. 1996, Capone et al. 1997). N_2-fixation rate is specifically enhanced when they are present as surface-dwelling aggregates (Carpenter 1983b, Paerl & Bebout 1988). Carpenter & Romans (1991) suggested that their ability to fix N_2 could be very important as a source of nitrogen in the tropical North Atlantic Ocean. *Trichodesmium* has now been recognized as an indispensable plankton component in tropical and subtropical oligotrophic oceans;

however, its biomass or biovolume has hardly been investigated because of the complicated colonial structure.

There are usually two types of colonial form in *Trichodesmium*: one is a separate filamentous form and the other is a fasciculated trichome form. The former is merely a uniserial array of individual cells, i.e. monofilament; cells are connected by transverse cell walls (Anagnostidis & Komárek 1988). On the other hand, the latter type of colony consists of a number of filaments, e.g. 10–30 trichomes in *Trichodesmium erythraeum* and 30–50 trichomes in *Trichodesmium thiebautii* (Nagasawa & Marumo 1967). Trichome filaments are sometimes strongly entangled with each other, especially in a large-sized colony, and hence the three-dimensional structure of fasciculated forms is too complicated to estimate the biovolume from normal microscopic observations. Owing to such technical difficulties, the standing crop of *Trichodesmium* has been expediently expressed as the number of trichome filaments in a given volume of water. Such a quantitative unit is, however, employed exceptionally in *Trichodesmium*, and is inconvenient for comparing with other plankton. Furthermore, since the trichome filament has a wide variation in its size even in the same population (e.g. 10–12 μm width$\times$60–750 μm length in *T. erythraeum* and 3–5 μm width$\times$1000–2000 μm length in *T. thiebautii*: Nagasawa & Marumo 1967), abundance

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values expressed as the number of filaments could be inaccurate and disadvantageous for quantitative ecological studies. In this study, biovolumes of *Trichodesmium* spp. were estimated through patient microscopic observations by applying the quantitative protargol staining method (Montagnes & Lynn 1993). Based on this basic ecological survey, the spatial distributions of *Trichodesmium*, both vertical and horizontal patterns, were quantitatively analyzed in spring in the East China Sea.

Materials and Methods

Trichodesmium spp. were collected in the eastern part of the East China Sea on a cruise of the T/S Kakuyo-maru from May 22 to 29, 1999. Vertical profiles were investigated at Stns 1, 2, 4, 6, 7 and 8 (Fig. 1), while horizontal profiles and temporal changes were traced along a line extending from continental shelf water (Stn 2) to Kuroshio water (Stn 6).

Vertical profile

Water was taken at 10 to 12 depth layers from the surface to near bottom depth or to 300 m depth using a rosette-multi-sampler attached to a CTD (Sea-Bird Electronics 11 plus). Surface water was collected by a bucket. Water temperature and salinity were simultaneously recorded with the CTD. Water samples in 100 ml aliquots were filtered through a GF/F filter immediately after sampling, and the chlorophyll *a* concentration was measured by a fluorescence technique (Parsons et al. 1984). Another 30 ml from each sample was used for the counting of coccoid cyanobacteria. They were fixed with glutaraldehyde at 0.5% final concentration and filtered through a pre-stained Nuclepore filter of 0.2 μm opening. Enumeration and size measurement were carried out under an epifluorescent microscope (Maclasaac & Stockner 1993).

Another 150 ml of each water sample was fixed for *Trichodesmium* spp. analysis with Bouin's solution at 10% final concentration. The fixed samples were brought back to the laboratory, stained and mounted into permanent slides with the quantitative protargol stain (QPS) method (Montagnes & Lynn 1993). *Trichodesmium* specimens of both the separate filamentous form and the fasciculated filamentous form were observed under a biological microscope with suitable magnifications (40–1000 x). Species identification was not carried out in this study because it depends on minute and subtle differences in trichome morphology (e.g. Umezaki 1974) and the differences were frequently indistinguishable.

Biovolume of the separate filamentous form was calculated from the length (l) and width (w) of a trichome filament on the assumption that the filament has an elongated cylindrical morphology ($0.25\pi w^2 l$). Biovolume of the fasciculated filamentous form, on the other hand, was estimated by totaling the biovolumes calculated for each individual filamentous component. If any of the trichome filaments were inextricably intertwined and the biovolume of each and every filament was unable to be determined, the biovolume of such a fasciculated colony was estimated as follows: a colony was divided imaginarily into seven parts with equal length along its long axis, the net area of each cross-section was estimated, biovolume of each part was calculated from the cross-sectional area and the part length, and finally the biovolumes of all seven parts were summed.

Horizontal profile and its temporal change

Repeated investigations were carried out at five stations arrayed along a line extending from continental shelf water (Stn 2) to Kuroshio water (Stn 6) for five days (May 24 to 28, 1999). Surface water was taken by a bucket at the stations every day. Temperature and salinity were simultaneously measured with an electric sensor (YSI Model-85).
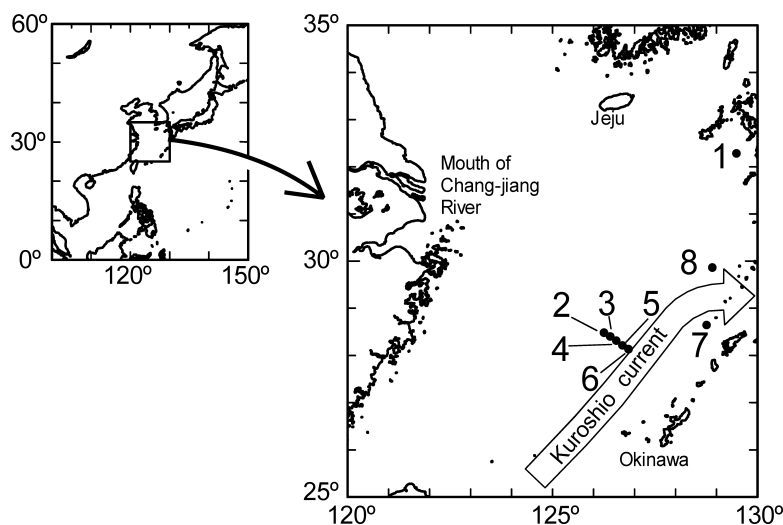
Fig. 1. Sampling stations occupied on the cruise of the T/S Kakuyo-maru from May 22 to 29, 1999. Position of the Kuroshio Current is cited from Japan Coast Guard (1999).

Chlorophyll *a* concentration and coccoid cyanobacterial biovolume were also measured according to the aforementioned methods.

Separate filamentous *Trichodesmium* was processed according to the QPS method (Montagnes & Lynn 1993), and its biovolume was estimated by the method outlined above. Fasciculated *Trichodesmium*, on the other hand, was processed by the following expedient method: two-liters of water was fixed with neutral formalin (2%) immediately after sampling, it was gently filtered through a Millipore® filter of 0.45 μm pore size, and *Trichodesmium* specimens on the filter were directly observed in the wet condition under a biological microscope with epi-illumination. To facilitate the investigation of numerous colonies, the biovolume of fasciculated colonies was approximated after deriving a regression equation between 'biovolume' (variable) and 'nominal colony volume' (independent variable). This 'nominal colony volume' is the volume of a colony outline, (colony width)^2 × colony length, and easily measured under a biological microscope. Thirty five fasciculated colonies were randomly selected at Stns 2–6 for the regression analysis, and they were carefully examined to obtain their biovolumes by the aforementioned method, as well as having their nominal colony volumes calculated. Since fasciculated *Trichodesmium* has a wide variation in colony size, they were, for convenience, divided into two size categories: one the small-sized forms shorter than 2 mm in colony length, and the other large-sized forms longer than 2 mm.

Results

Vertical profiles

Vertical profiles of water temperature showed broadly similar patterns among the six stations: higher in the surface or near surface layer, and lower in the deeper layers (Fig. 2). The southernmost station (Stn 6) was strongly influenced by the Kuroshio Current according to the Japan Coast Guard (1999), and it had the maximum temperature (26.4°C at 25 m depth) found in this study. The northernmost station (Stn 1), which is far from the Kuroshio Current, had the lowest temperature.

Salinity ranged from 33.27 to 34.85 psu (Fig. 3). The westernmost station (Stn 2), which is in continental shelf water, showed lower values especially in the shallower layers, while the south-eastern station (Stn 7), which is in the east of the Kuroshio Current, had higher salinity throughout the water column.

Chlorophyll *a* exhibited a sub-surface maximum pattern at every station (Fig. 4). The northernmost station (Stn 1) showed a remarkable peak of 0.97 μg L^{-1} at 30 m depth, while the other stations had smaller peaks of 0.42–0.59 μg L^{-1} at 30–75 m depth.

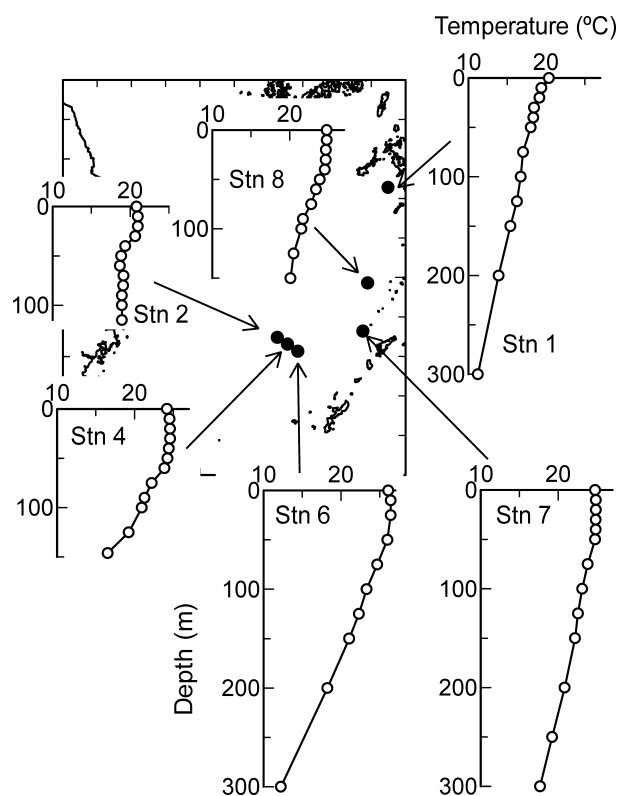Coccoid cyanobacteria occurred at all stations and were most prevalent in the sub-surface layer (Fig. 5). According

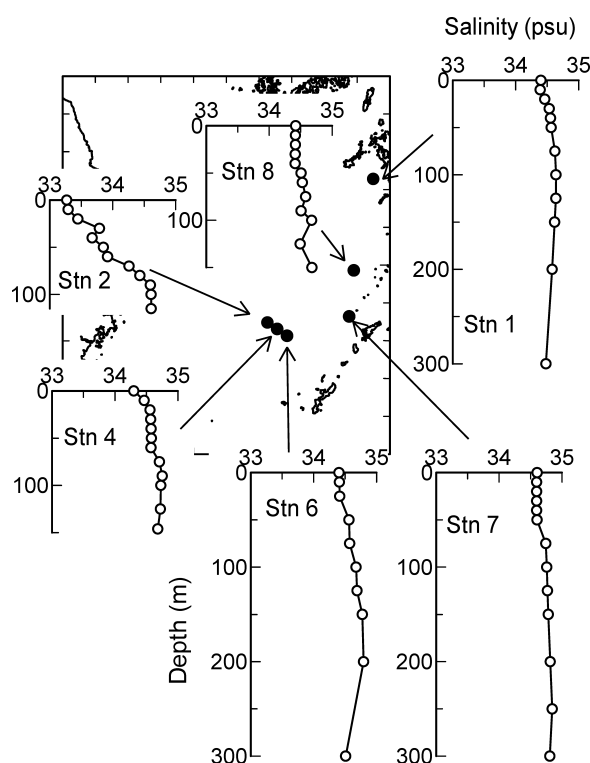**Fig. 2.** Vertical profiles of water temperature at Stns 1, 2, 4 and 6–8.

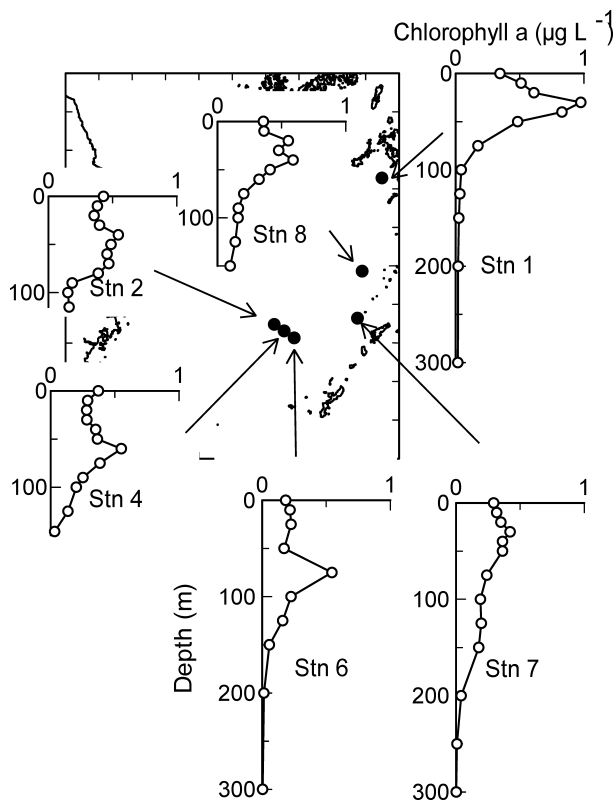**Fig. 3.** Vertical profiles of salinity at each station.

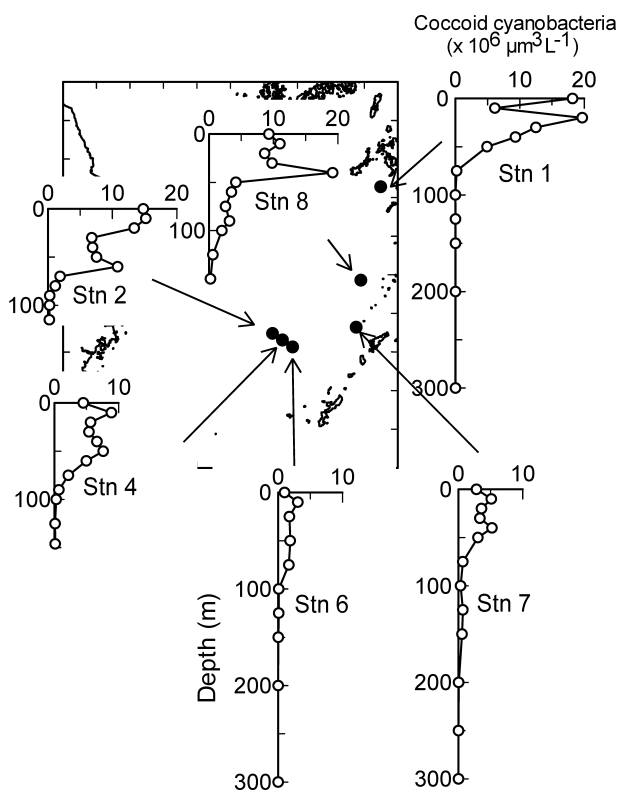**Fig. 4.** Phytoplankton chlorophyll *a* over depth at each station.

Fig. 5. Biovolume of coccoid cyanobacteria over depth at each station.
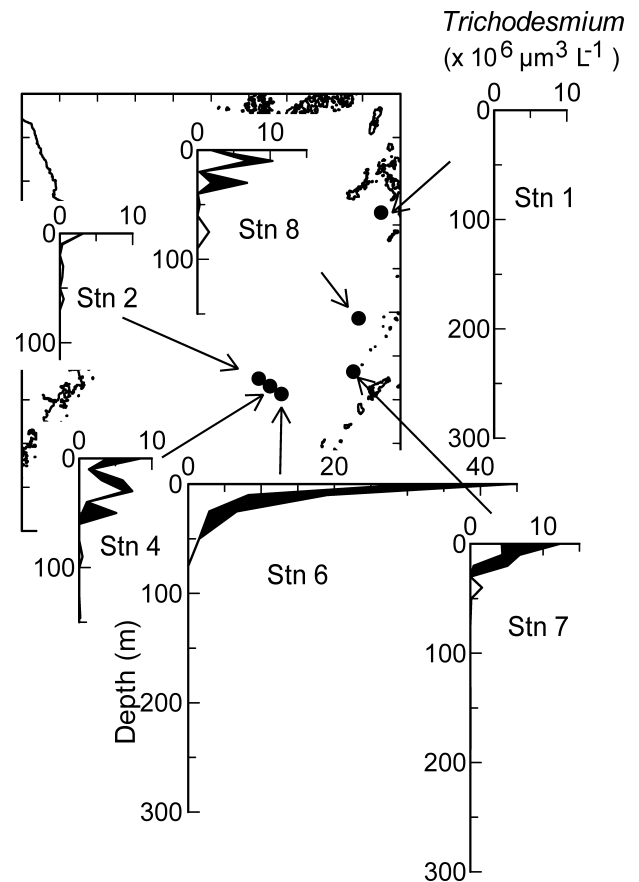
Fig. 6. Biovolume of *Trichodesmium* spp. over depth at each station. Dark area indicates fasciculated form and white area the separate filamentous form.

to Maclassac & Stockner (1993), most of them were *Synechococcus* spp. The sub-surface maximum was 8.9×10^6–$2.0 \times 10^7 \, \mu m^3 \, L^{-1}$ in biovolume, and this layer occurred between 10 and 40 m depth. The northern stations (Stns 1 and 8) exhibited higher biomass in the maximum layer, while the southern stations (Stns 6 and 7) had lower biomass throughout the water column. Integrated biovolume in the upper 115 m of the water column ranged from 1.7×10^{11} to $7.3 \times 10^{11} \, \mu m^3 \, L^{-2}$; lower values were observed in the middle or east of the Kuroshio Current, while higher values occurred in the north or west of the current.

Trichodesmium spp. were abundant in the surface layer both in the separate and fasciculated forms (Fig. 6), and the layer of maximum abundance was shallower than the chlorophyll *a* maximum layer, though it was not detected at the northernmost station (Stn 1). The southernmost station (Stn 6), positioned in the Kuroshio Current, exhibited an exceptionally large *Trichodesmium* biovolume at the surface: $3.1 \times 10^7 \, \mu m^3 \, L^{-1}$ for the separate filamentous form and $1.3 \times 10^7 \, \mu m^3 \, L^{-1}$ for the fasciculated filamentous form. Integrated biovolume in the upper 115 m of the water column varied widely among the six stations; it ranged from 0 (Stn 1) to $6.3 \times 10^{11} \, \mu m^3 \, m^{-2}$ (Stn 6). The separate filamentous form dominated over fasciculated filamentous form at

many stations (Stns 2, 4, 6 and 8), while the latter was slightly predominant at Stn 7.

Horizontal profile and temporal changes

Water temperature and salinity in the surface layer showed distinct gradients along a line extending from Stn 2 to Stn 6 (Fig. 7a, b). These two parameters were higher in the eastern part (>26°C and >34.3 psu) and lower in the western part (<23°C and <33.9 psu); their standard deviations (SDs) on a spatial basis, i.e. SDs among the five observations obtained at the respective stations (Stns 2–6) on

each day, were 1.6–2.3°C and 0.22–0.50 psu, respectively (Table 1). Temporal variations of these environmental parameters, on the other hand, were trivial during the five day period; SDs on a temporal basis, i.e. SDs among the five observations obtained on the respective days (May 24–28) at each station were 0.15–1.14°C and 0.09–0.26 psu, respectively.

Horizontal profiles of chlorophyll *a* exhibited a different pattern to those of surface temperature or salinity in lacking temporal stability at each station, while it also exhibited spatial gradients occasionally in the easterly-westerly direction (Fig. 7c). Chlorophyll *a* concentration ranged from
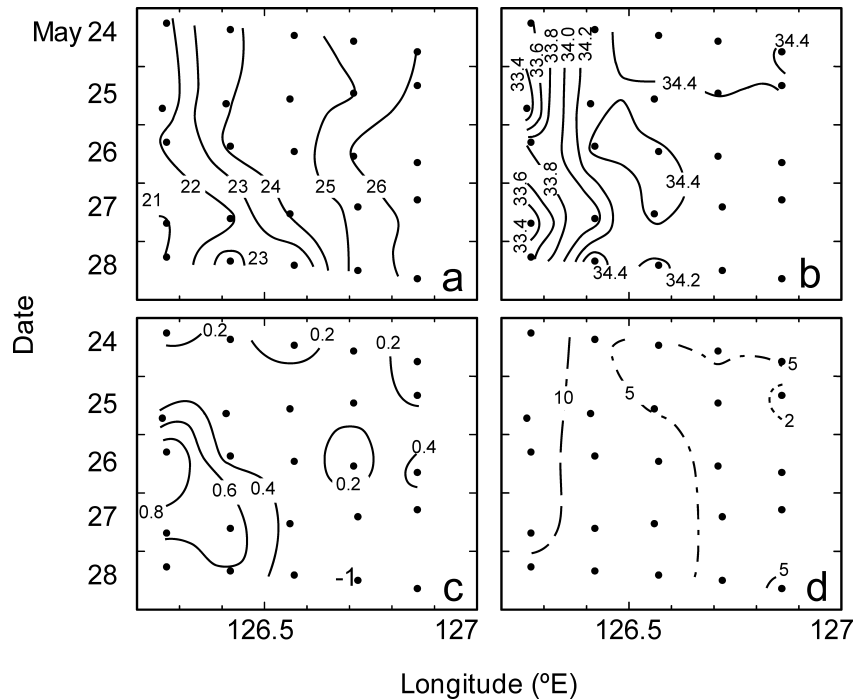
Fig. 7. Horizontal profiles of water temperature (°C) (a), salinity (b), phytoplankton chlorophyll *a* (μg L^{-1}) (c), and coccoid cyanobacterial biovolume ($\times 10^6$ μm^3 L^{-1}) (d) between Stn 2 and Stn 6 from May 24 to 28, 1999.

Table 1. Standard deviation (SD) and coefficient of variation (CV) of temperature (°C), salinity (psu), chlorophyll *a* (μg L^{-1}), coccoid cyanobacteria (μm^3 L^{-1}), and *Trichodesmium* spp. (μm^3 L^{-1}). Data on spatial basis are five observations obtained at Stations 2–6 on each day, and those concerning the temporal basis are the five observations obtained on respective days (May 25–28) at each station.

Parameter	Temporal basis		Spatial basis	
	SD	CV (%)	SD	CV (%)
Temperature	0.15–1.14 (°C)		1.63–2.23 (°C)	
Salinity	0.05–0.26 (psu)		0.22–0.50 (psu)	
Chlorophyll *a*	0.10–0.33 (μg L^{-1})	36–62	0.07–0.36 (μg L^{-1})	32–82
Coccoid cyanobacteria	1.0×10^6–3.7×10^6 (μm^3 L^{-1})	16–55	2.1×10^6–6.3×10^6 (μm^3 L^{-1})	34–88
Trichodesmium spp.				
Total	1.1×10^7–4.6×10^7 (μm^3 L^{-1})	60–170	5.8×10^6–3.8×10^7 (μm^3 L^{-1})	44–110
Separate filamentous form	5.6×10^6–2.9×10^7 (μm^3 L^{-1})	93–160	2.0×10^6–2.6×10^7 (μm^3 L^{-1})	49–160
Small fasciculated form	3.5×10^6–1.6×10^7 (μm^3 L^{-1})	85–180	2.3×10^6–1.5×10^7 (μm^3 L^{-1})	67–220
Large fasciculated form	2.0×10^6–4.5×10^6 (μm^3 L^{-1})	85–140	9.2×10^5–3.5×10^6 (μm^3 L^{-1})	37–170

0.05 μg L^{-1} to 1.02 μg L^{-1}; it was higher in the western part on May 26 to 27 and lower in the eastern part throughout the five days. The temporal variation was almost comparable to the spatial variation; SD and coefficient of variation (CV) on a temporal basis were 0.10–0.33 μg L^{-1} and 32–62%, and those on a spatial basis were 0.10–0.36 μg L^{-1} and 32–82 % (Table 1).

Coccoid cyanobacteria exhibited a remarkable gradient in their biovolume in the easterly-westerly direction: higher in the western part and lower in the eastern part (Fig. 7d). The spatial variation, 2.1×10^6–6.3×10^6 μm^3 L^{-1} in SD and 34–88% in CV, was rather larger than the temporal variation, 1.0×10^6–3.7×10^6 μm^3 L^{-1} in SD and 13–55% in CV (Table 1).

Fasciculated *Trichodesmium* colonies were frequently observed at Stns 2–6, there was a functional relationship between nominal colony volume (x) and biovolume (y) on both logarithmic scales, and the regression equation could be expressed as $y = 10^3 \times x^{0.703}$; $r^2 = 0.89$, $P < 0.01$ (Fig. 8). This equation was obtained from the data sets of 35 colonies of fasciculated *Trichodesmium* spp. picked out randomly from surface water at Stns 2–6. The size of the specimens ranged from 1.2×10^4 to 5.9×10^8 μm^3 in nominal colony volume and from 9.1×10^3 to 2.3×10^7 μm^3 in biovolume.

Trichodesmium biovolume ranged from 2.3×10^5 to 1.1×10^8 μm^3 L^{-1}, and it varied strongly both in its spatial and temporal distributions (Fig. 9a); SD and CV on a spatial basis were 1.1×10^7–4.6×10^7 μm^3 L^{-1} and 60–170%, and on a temporal basis were 5.8×10^6–3.8×10^7 μm^3 L^{-1}

and 44–110% (Table 1). The separate filamentous form and small fasciculated forms were predominant when the total *Trichodesmium* biovolume exhibited a sporadic maximum (Stn 2 on May 26). The large-sized fasciculated form, on the other hand, was dense in the eastern part on May 27 or 28 (Fig. 9d), when the total *Trichodesmium* biovolume was not so large. Temporal variation was particularly remark-
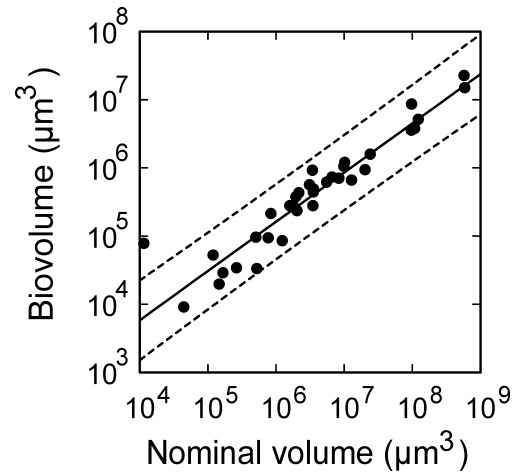
Fig. 8. Relation between nominal colony volume (x) and biovolume (y) in fasciculated *Trichodesmium* spp. Nominal colony volume is the volume calculated from colonial outline, (colony width)^2 × colony length. Biovolume is the total of every filament biovolume in a colony. Solid line indicates the regression equation of $y = 10^3 \times x^{0.703}$, $r^2 = 0.89$, and dashed lines do 95% confidence limits.
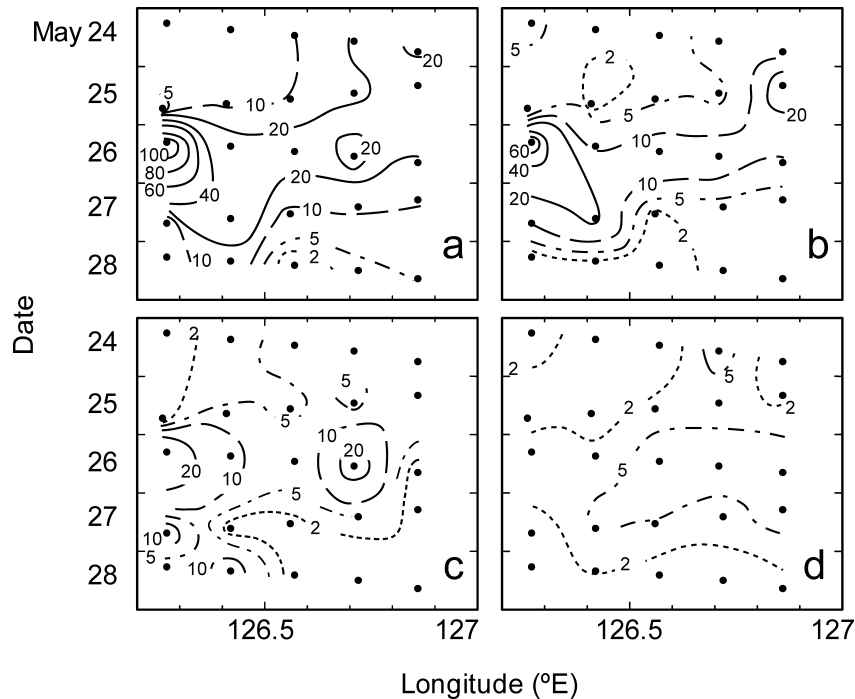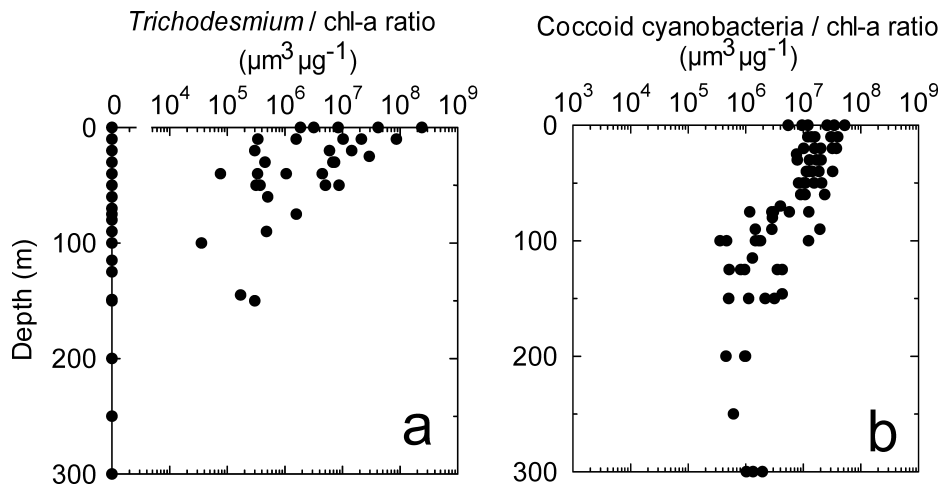
Fig. 9. Horizontal profile of *Trichodesmium* spp. ($\times 10^6$ μm^3 L^{-1}) between Stn 2 and Stn 6 from May 24 to 28, 1999. (a) Total *Trichodesmium*, (b) separate filamentous form, (c) small fasciculated form shorter than 2 mm in colony length, and (d) large fasciculated form longer than 2 mm in colony length.

Fig. 10. *Trichodesmium*/chlorophyll *a* ratio ($\mu m^3 \, \mu g^{-1}$) (a) and coccoid cyanobacteria/chlorophyll *a* ratio ($\mu m^3 \, \mu g^{-1}$) (b) over depth. Data are compiled from Stns 2, 4 and 6–8 for *Trichodesmium*/chlorophyll *a* ratio, and from Stn 1, 2, 4 and 6–8 for coccoid cyanobacteria/chlorophyll *a* ratio.

able for every form; CV on a temporal basis was 93–160% in separate filamentous forms, 85–180% in small fasciculated forms and 85–140% in large fasciculated forms, and these values exceeded those of chlorophyll *a* (36–63%) or coccoid cyanobacteria biomass (16–55%) (Table 1).

Discussion

Trichodesmium spp. biomass was not correlated to any visible degree with phytoplankton chlorophyll *a* (Fig. 10a), while the coccoid cyanobacteria to chlorophyll *a* ratio was almost constant from the surface layer to 50 m depth (Fig. 10b). *Trichodesmium* has a number of minute gas vesicles in each cell (e.g. Walsby 1972, 1987), and hence it has enough positive buoyancy to move upward in the water column (Carpenter 1983a, Capone & Carpenter 1999). Such accumulation through floating might be significant in this study, and it would be more so under gently stratified sea conditions around the boundary area and the Kuroshio Current. Since such stratified conditions limit the upward supply of inorganic nutrients from the deeper layers, the positive buoyancy produced by gas vesicles should cause upward transportation of nutrients in oligotrophic conditions; active *Trichodesmium*, the gas vacuoles of which are functional, does not stay constantly in the nutritious sub-surface layer under stratified sea conditions. The survival strategy of *Trichodesmium* around the boundary area and the Kuroshio Current in spring, therefore, might not strongly depend on growth under eutrophic conditions but on adaptation to severe nutritional conditions in the surface layer.

Water density decreased gradually toward the surface: $1.024 \, g \, cm^{-3}$ at 100 m depth to $1.022 \, g \, cm^{-3}$ at the surface in the Kuroshio Current and $1.025 \, g \, cm^{-3}$ at 100 m depth to $1.023 \, g \, cm^{-3}$ at the surface around the boundary area. Positive buoyancy of *Trichodesmium* therefore would be slightly reduced on the way to the surface layer. Even

though this reduction in buoyancy occurred in this study, its effect on the positive buoyancy of *Trichodesmium* was too small to interfere with the intensity of surface distribution.

Coccoid cyanobacteria, on the other hand, do not have positive buoyancy because they lack gas vesicles. They do not have swimming apparatus either, and their cell size is too small for substantial sinking in the water column (Fogg 1987). Owing to their non-motile character, their dense distribution in the sub-surface layer (Fig. 5) must be due mainly to active binary division accompanied by photosynthetic production; the layer of greatest abundance should be the most productive layer. This concentrative process is different from the accumulating process of *Trichodesmium* in the surface layer. Since inorganic nutrients increased gradually with depth around the boundary area and the Kuroshio Current (Nagasaki Marine Observatory 1999, Furuya et al. 2003), the survival strategy of coccoid cyanobacteria might be dependent on their photosynthetic growth under more eutrophic conditions.

Trichome filaments, either in separate form or in fasciculated form, are far larger than coccoid cyanobacterial cells; the former can be directly grazed upon by metazoan organisms such as the harpacticoid copepod *Macrosetella gracilis* and pelagic fishes (Carpenter 1983a), while the latter are generally grazed on by small-sized unicellular organisms such as heterotrophic nanoplankton (e.g. Fenchel 1987). In this study, *Trichodesmium* spp. occurred intensively in the surface layer around the Kuroshio Current, and their biovolume sometimes exceeded coccoid cyanobacterial biovolume (Fig. 6). Coccoid cyanobacteria, on the other hand, tended to exhibit maximum biovolume in the sub-surface layer (Fig. 5), and their biovolume frequently exceeded the *Trichodesmium* biovolume in this layer. From these contrasting vertical distributions, the major grazers may differ with depth even within the same water column; large-sized grazers may be important in the surface layer, while small-

sized grazers might be important in the sub-surface layer.

Trichodesmium spp. occurring in the surface layer would inevitably be subjected to various physical processes. Wind-driven force and convergent-divergent currents are possible causes of the uneven and variable pattern in their horizontal distribution (Table 1). When trichome filaments are at high densities in the surface layer, they must frequently collide with each other. Furthermore, considering their production of slime on the surface (Borstad & Borstad 1977, Carpenter 1983a, Anagnostidis & Komárek 1988), such encounters might promote the adhesion and aggregation of trichome filaments. Frequent repetition of these processes might result in the enlarging of *Trichodesmium* colonies. Adopting the spatial segregation hypothesis, i.e. N_2-fixation is carried out in the central part of a fasciculated colony and oxygenic photosynthesis is done on the outskirts (Fogg 1974, Carpenter & Price 1976, Paerl 1999, 2000), such enlargement might entail significant morphological adaptation to promote N_2-fixation. Although this hypothesis is not always universally supported under artificial culture conditions (Ohki & Fujita 1988), it is supported by the horizontal profile observed in this study: the biovolume of large-sized colonies was relatively higher on the oligotrophic Kuroshio side (Fig. 9). Furthermore, large-sized colonies exhibit stronger nitrogenase activity than disintegrated ones (Saino & Hattori 1980) and separate trichomes cease to fix N_2 under the high irradiance levels experienced in oceanic surface water (Prufert-Bebout et al. 1993, Pearl 1994). Therefore, intensive distribution in the surface layer, enlargement of colony size, and spatial segregation of N_2-fixation might be the magic trinity for the survival strategy of *Trichodesmium* around the Kuroshio Current area.

Although the horizontal distribution of *Trichodesmium* spp. changed strongly over the course of time, it showed different patterns between the large-sized fasciculated form and the other form. The former was more abundant on the Kuroshio side, while the small-sized form or the separate filamentous form was more abundant on the continental shelf side (Fig. 9). Fogg (1982) suggested that colony size in *Trichodesmium* is closely related to the process of development: the separate filamentous form is in the early developmental stage, and the large-sized fasciculated form is more developed. Furthermore fortuitous collision among trichome filaments could gradually enlarge the size of colonies as mentioned above. Considering the effect of this perfectly feasible enlargement process, the contrasting distributional patterns observed in this study might reflect the eastward transport of *Trichodesmium* from continental shelf water to Kuroshio Current water. *Trichodesmium* stocks in the early developmental stage might be rich on the continental side, and some might be transported toward the main stream of the Kuroshio Current in spring in the East China Sea.

In this hypothetic transport scheme, *Trichodesmium* coming from continental shelf water would soon be exposed for a certain period to the more oligotrophic Kuroshio Current (Nagasaki Marine Observatory 1999, Furuya et al. 2003, Shimizu et al. 2004), and hence it should better enhance potential N_2-fixation as severe nutritional conditions are encountered. Considering again that the spatial segregation hypothesis (Fogg 1974, Carpenter & Price 1976, Paerl 1999) may explain distributions in this area, enlargement of fasciculated colonies might be a reasonable morphological adaptation to cope with horizontal transport from continental shelf waters to the more oligotrophic Kuroshio Current. Although this transport has not been observed directly in the field, it is consistent with the observation that the integrated biovolume of fasciculated forms in the water column was much greater than that of the separate filamentous form on the east side of the Kuroshio Current (Stn 7, Fig. 6). Furthermore, it may also explain the widespread biased distribution of *Trichodesmium* observed along the western boundary current system extending from the North Equatorial Current to the Kuroshio Current, i.e. *Trichodesmium* occurring in large numbers toward the lower reaches of the stream after being influenced by eutrophic coastal water (Nagasawa & Marumo 1967).

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