Vol. 133: 263–273, 1996 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published March 28

Role of *Trichodesmium* spp. in the productivity of the subtropical North Pacific Ocean

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ABSTRACT: The concentrations of filamentous diazotroph *Trichodesmium* spp., present as free trichomes and in colonial assemblages, were measured at approximately monthly intervals at Stn ALOHA (22° 45' N, 158° 00' W) between October 1989 and December 1992. The average abundance of filaments in the upper 45 m of the water column was highly variable ranging from 1.1 to 7.4×10^4 trichomes m^{-3} and from 0.02 to 1.4×10^2 colonies m^{-3}. Colonies were composed, on average, of 182 filaments accounting for 12% of total (free filament plus colonies) *Trichodesmium* biomass. Low densities of single trichomes were associated with, but not restricted to, deep mixing events and winter periods. During 1991 and 1992 the concentration of *Trichodesmium* spp. present in the water column increased relative to the pre 1991 observations. This increase coincided with increases in photosynthetic carbon assimilation and in the molar ratio of N:P of suspended particulate matter in the upper 45 m of the water column. However, the change in *Trichodesmium* biomass alone does not account for the change observed in autotrophic carbon assimilation and elemental biomass composition. *Trichodesmium* spp. comprised, on average, 18% of the chlorophyll *a*, 4% of the photosynthetic carbon assimilation, 10% of the particulate nitrogen and 5% of the particulate phosphorus. We also estimate that *Trichodesmium* dinitrogen fixation accounted for, on average, at least 27% of the new production at this study site. These observations, combined with primary production experiments conducted on isolated colonies, suggest that phytoplankton production is enhanced due to the release of NH_4^+ and dissolved organic nitrogen by *Trichodesmium* spp. during episodes of nitrogen fixation.

KEY WORDS: *Trichodesmium* · Nitrogen fixation · Nutrients · Productivity · Time-series

INTRODUCTION

Spatial and temporal distributions of the diazotrophic (nitrogen-fixing) cyanobacterium *Trichodesmium* have been extensively studied in the eastern section of the tropical North Atlantic Ocean and Caribbean Sea (Carpenter & Price 1976, Carpenter & Romans 1991), in the Indian Ocean (Devassy et al. 1978), in the East China Sea (Marumo & Asaoka 1974a), and in the Pacific Ocean (Marumo & Asaoka 1974b, Marumo & Nagasawa 1976). The presence of *Trichodesmium* has also been frequently reported in oligotrophic marine environments in the context of phytoplankton bloom phenomena (Bowman & Lancaster 1965, Dupuoy et al. 1988, Karl et al. 1992). Nevertheless, the importance of *Trichodesmium* in marine ecosystems and the marine nitrogen cycle remains a matter of debate (Carpenter 1983, Codispoti 1989, Capone et al. 1992).

Field observations have shown that *Trichodesmium* colonies become abundant in surface waters of tropical and subtropical marine ecosystems during periods of calm weather (Steven & Glombitza 1972, Carpenter & Price 1976, Bryceson & Fay 1981, Karl et al. 1992). These colonies of *Trichodesmium* cells support an entire localized ecosystem including epiphytic heterotrophic bacteria (Carpenter & Price 1976, Borstad & Borstad 1977, Paerl et al. 1989), diatoms (Borstad & Borstad 1977, Bryceson & Fay 1981), and hydrozoans (Geiselman 1977, Borstad & Brinckmann-Voss 1979). There is also evidence that the survival rate of larval stages of the copepod *Macrosetella gracilis* (Dana) increases when *Trichodesmium* is present (Bjornberg 1965, Calef & Grice 1966, Roman 1978). *Trichodesmium*

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also undergoes rapid lysis when senescent (Van Baalen & Brown 1969, Borstad & Borstad 1984). Devassy et al. (1979) concluded from the succession following a *Trichodesmium* bloom that rapid cell structure degradation resulted in an increase of inorganic nutrients in the water column, presumably the result of a rapid and efficient microbial recycling of organic constituents following cell lysis. Furthermore, recent studies indicate that, even under healthy conditions, *Trichodesmium* releases NH_4^+ (Prufert-Bebout et al. 1993) and dissolved organic nitrogen (Capone et al. 1994, Glibert & Bronk 1994) during the process of nitrogen fixation. These observations suggest that fluctuations in the abundance of *Trichodesmium* in the pelagic marine environment may affect both the taxonomic and chemical composition of the ambient plankton community. In the subtropical North Pacific gyre, where nitrogen is generally considered to be the production rate limiting nutrient controlling productivity, nitrogen fixation and its release in the form of ammonium, or other utilizable forms of nitrogen, may be an important source of 'new' nutrient for phytoplankton otherwise incapable of direct assimilation of gaseous nitrogen.

Most field studies of *Trichodesmium* have concentrated on either the spherical or fusiform colony morphologies of this genus in spite of the fact that Marumo & Asaoka (1974a) and Marumo & Nagasawa (1976) have reported that a major fraction of *Trichodesmium* biomass occurs in the form of single trichomes. Historically, single trichome morphology has been considered less important in the context the marine nitrogen cycle because it was thought that nitrogen fixation occurred only in sub-oxic microenvironments found in the center of selected colonies (Carpenter & Price 1976, Bryceson & Fay 1981, Paerl & Bland 1982, Paerl & Prufert 1987, Paerl & Bebout 1988, but see Saino & Hattori 1982). This view has recently been challenged as a result of studies of nitrogen fixation performed in *Trichodesmium* cultures (Ohki & Fujita 1988, Prufert-Bebout et al. 1993) and by enzymatic analyses of natural *Trichodesmium* assemblages (Capone et al. 1990, Carpenter et al. 1990, Bergman & Carpenter 1991). This evolution of thinking about the potential role of free trichomes in nutrient cycles has prompted renewed speculation about the quantitative importance of oceanic nitrogen fixation processes.

The Hawaiian Ocean Time-series (HOT) program is designed to characterize oligotrophic habitat and ecosystem variability in the water column at Stn ALOHA (A Long-term Oligotrophic Habitat Assessment, 22° 45' N, 158° 00' W). This program provides an optimum background data set to study the effects that changes in *Trichodesmium* abundances may have in the biochemical characteristics of the euphotic zone. In this paper we report results of the study of the distribution of *Trichodesmium* in the euphotic zone of Stn ALOHA between August 1989 and December 1992. We also analyze the variability of *Trichodesmium* biomass in the upper 45 m of the euphotic zone as it relates to changes in the elemental ratio of particulate matter and primary production over similar depth ranges.

METHODS

The Hawaiian Ocean Time-series (HOT) program investigators sample the water column of Stn ALOHA at approximately monthly intervals as part of the United States-Joint Global Ocean Flux Study (US-JGOFS; Karl & Winn 1991, Karl & Lukas in press). As part of this program, water column suspended particulate carbon (PC), particulate nitrogen (PN), particulate phosphorus (PP), and pigments were measured during each cruise. Water samples (4 to 10 l), collected with a 24-position rosette water sampler equipped with 12 l polyvinylchloride (PVC) bottles and SeaBird CTD, were pre-screened through a 202 µm mesh and filtered onto combusted 25 mm GF/F Whatman filters for PC and PN analyses, or onto combusted and acid washed 25 mm GF/F filters for PP analyses. PC and PN were measured simultaneously in a Perkin-Elmer model 2400 CHN analyzer. PP was quantified spectrophotometrically. For this analysis samples were combusted (450 to 500°C), acidified (0.5 N HCl at 90°C for 90 min) and color proportional to the concentration of phosphorus in solution was developed by adding a mixed reagent freshly prepared (10 ml ammonium molybdate, 25 ml 5 M sulfuric acid, 10 ml 5.4% ascorbic acid and 5 ml potassium antimony tartrate; Strickland & Parsons 1972). Particulate samples for pigment analyses were collected and quantified using the chromatographic method described by Letelier et al. (1993).

In order to assess the temporal and vertical distributions of *Trichodesmium* colonies, horizontal net tows of discrete subsurface layers were obtained using an opening-closing plankton net (Omori 1965; 50 cm in mouth diameter, 270 cm in filtering cloth length and 100 µm in mesh size, 9 cm bucket diameter with 60 µm mesh size) between March 1990 and October 1991. When not counted at sea, samples were preserved in 5% buffered formalin and analyzed at our shore-based laboratories. A flow meter (General Oceanics) was mounted at the mouth of the net to calculate the total volume of water filtered during each tow and convert colony numbers to densities. A typical horizontal tow sampled approximately 4.3 m^3 min^{-1} and lasted 10 min. Vertical net tows (0 to 100 m; 16 m^3 total) were also performed between May 1990 and October 1991 to com-

pute an independent estimate of the depth-integrated concentration of colonies.

To assess the elemental (PC, PN, PP) and pigment compositions of *Trichodesmium* at Stn ALOHA, colonies from net tow samples were isolated using a Pasteur pipette, rinsed and vortexed in 50 ml filtered seawater to produce a suspension of single trichomes. Subsamples were transferred onto combusted GF/F filters, as above. Triplicate samples (>10 colonies per filter) were analyzed for each determination.

To examine the natural distribution of single trichomes, seawater was collected at discrete depths using the rosette water sampler described above. Water samples (4 to 10 l from each depth) were gently filtered onto Nitex mesh (10 µm mesh size). The mesh was back rinsed with 50 ml filtered seawater to resuspend the trichomes after manual removal of colonies, if present. This suspension was then filtered onto 0.8 µm 25 mm Nuclepore filters prestained with irgalan black and mounted on microscope slides. Trichomes were enumerated by epifluorescence microscopy based on phycoerythrin autofluorescence. To avoid overestimating the quantity of single trichomes in a sample as a result of filament breakage during processing, only apical cells were enumerated rather than total trichomes (intact filaments have shaped apical cells at either end). The abundance of single trichomes reported here corresponds to the number of apical cells divided by 2.

The elemental (PC, PN, PP) composition of single trichomes was studied on 2 separate occasions. A total of 8 replicates of 10 l each collected at 45 m depth were screened onto 10 µm mesh as described above. All replicates were combined into a single 500 ml pooled sample from which five 100 ml aliquots were drawn. One of the samples was used for microscopic enumeration, 1 for PP, 1 for pigment and 2 for PC/PN.

The abundance of trichomes found in colonial morphology was compared to the abundance of single trichomes by counting the number of filaments per colony. Triplicate 60 ml samples of filtered seawater containing 20 to 30 hand-picked colonies per sample were vortexed until no visible colonies remained in the suspension (5 to 30 min). From each sample, triplicate 5 ml subsamples were withdrawn and filaments were enumerated as described above.

During March 1990 and August 1991, primary productivity experiments were performed in conjunction with the standard primary productivity incubations of the HOT program to assess the contribution of *Trichodesmium* colonies to photoautotrophic production at Stn ALOHA. Colonies were collected at night from 5, 25, 45 and 75 m and transferred into filtered seawater corresponding to the respective sampling depth. All colonies were observed under a stereoscopic microscope to select only those that appeared to be intact and free of copepods. Once the regular primary production experiment had been deployed (see Letelier et al. in press), the remaining water was used to fill triplicate 500 ml polycarbonate incubation bottles for each water depth. One *Trichodesmium* colony was added to each bottle. This amounted to an increase in chlorophyll *a* (chl *a*) of approximately 100%. Triplicate polycarbonate bottles containing filtered 0.2 µm sterile filtered seawater were also inoculated with 3 colonies each. $NaH^{14}CO_3$ (final activity = 0.1 µCi ml^{-1}) was used as radiotracer to measure carbon assimilation. The samples were incubated on deck from approximately dawn to dusk under *in situ* simulated light and temperature conditions.

To correlate *Trichodesmium* abundances measured during each cruise with antecedent weather conditions, the sea surface temperatures (SST) recorded at hourly intervals by the National Data Buoy Center (NDBC) buoy no. 51001 (23°24'N, 162°18'W) were analyzed. From these hourly averages, we calculated a relative stability index based on the amplitude of the diel SST cycle. This approach assumes that the diel SST warming cycle, due to the local heating and cooling of the sea surface, increases in amplitude under calm weather conditions as a direct result of a decrease in turbulent mixing. Hence, a large daily amplitude in the diel SST cycle indicates stratification of the upper water column, while a low amplitude indicates a well-mixed water column (Karl et al. 1995).

RESULTS

The estimated densities of *Trichodesmium* colonies and single trichomes at Stn ALOHA are highly variable (Fig. 1). The concentration of single trichomes averaged over the upper 45 m of the water column ranges from 1.1 to 8.4 × 10^4 filaments m^{-3} ($\bar{x}$ = 4.6 × 10^4 filaments m^{-3}, s = 2.3 × 10^4 filaments m^{-3}, n = 22; Fig. 2B). Colony densities averaged over the same depth intervals ranged between 2 and 140 colonies m^{-3} ($\bar{x}$ = 31 colonies m^{-3}, s = 41 colonies m^{-3}, n = 11; Fig. 2B). Colony size also varied considerably in single samples (<10 to 372 filaments $colony^{-1}$). Nevertheless, the mean size between cruises varied only 85% (132 to 241 filaments $colony^{-1}$, $\bar{x}$ = 182 filaments $colony^{-1}$, s = 23 filaments $colony^{-1}$, n = 11).

Although the size of filaments covered a large range (6 to >250 cells $trichome^{-1}$), the average size for both morphologies (single trichomes and colonies) was similar (approximately 100 cells $trichome^{-1}$). *Trichodesmium* cells in colony morphology, therefore, comprised 12% (s = 11%, n = 11) of the total *Trichodesmium* biomass.

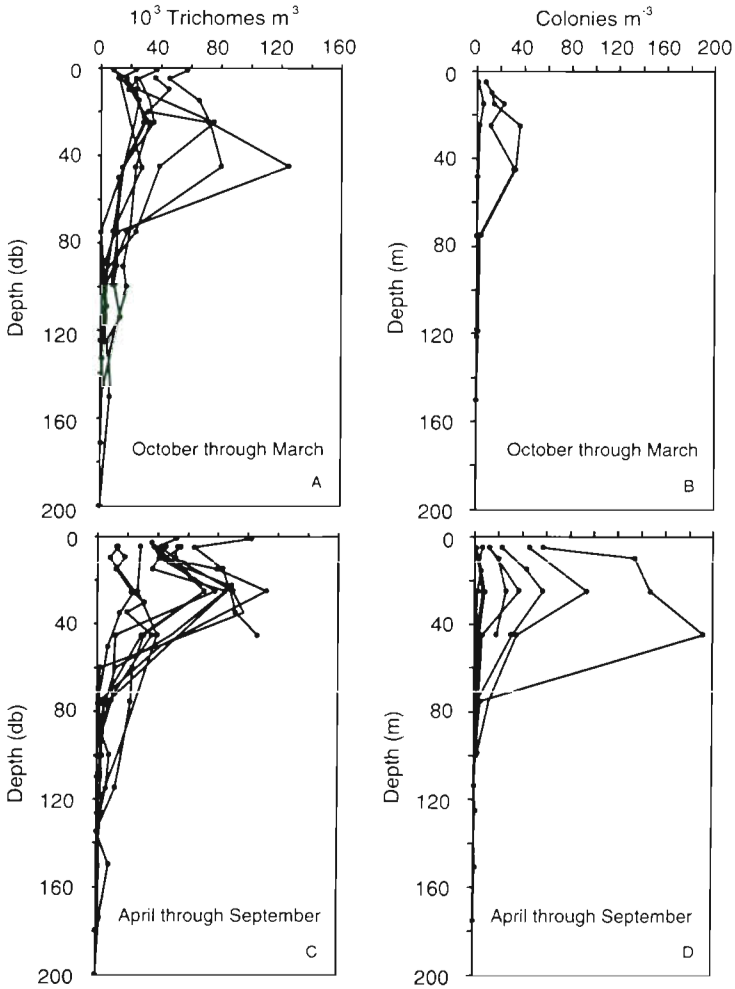

Fig. 1. *Trichodesmium* spp. Vertical distribution of single trichomes (A, C) and colonies (B, D) at Stn ALOHA between October 1989 and December 1992

The slope of a linear regression (model II geometric mean) between integrated colony concentration calculated from 0 to 100 m vertical net tows and from samples collected at discrete depths (horizontal net tows) was not different from 1.0 (95% confidence limits = 0.84 and 1.06, $R^2 = 0.85$, n = 9; Fig. 3) indicating that our sampling procedures were accurate. This result suggests that the reconstruction of the depth distribution of colonies from discrete depth samples is a valid representation of the vertical structure of the colony distribution for the water column.

The vertical distribution of both the filament and colony morphologies displays a subsurface maximum located between 20 and 50 m in all cruises where the mixed-layer depth is <50 m (Fig. 1). However, this subsurface maximum disappears when the mixed-layer deepens and exceeds a depth of 50 m. A near surface maximum of single trichomes (2 to 5 m) is also observed in 40% of the cruises and, although an accumulation of colonies at the sea surface was visually observed from the ship on 2 occasions (August 1991 and June 1992), this feature was not visible at the time of sampling and was not resolved by the sampling procedures employed.

The abundance of single trichomes in the first 45 m of the water column does not correlate with the average diel SST amplitude calculated from the NDBC buoy no. 51001. Several running averages, from 1 to 14 d intervals, were performed to assess the cor-

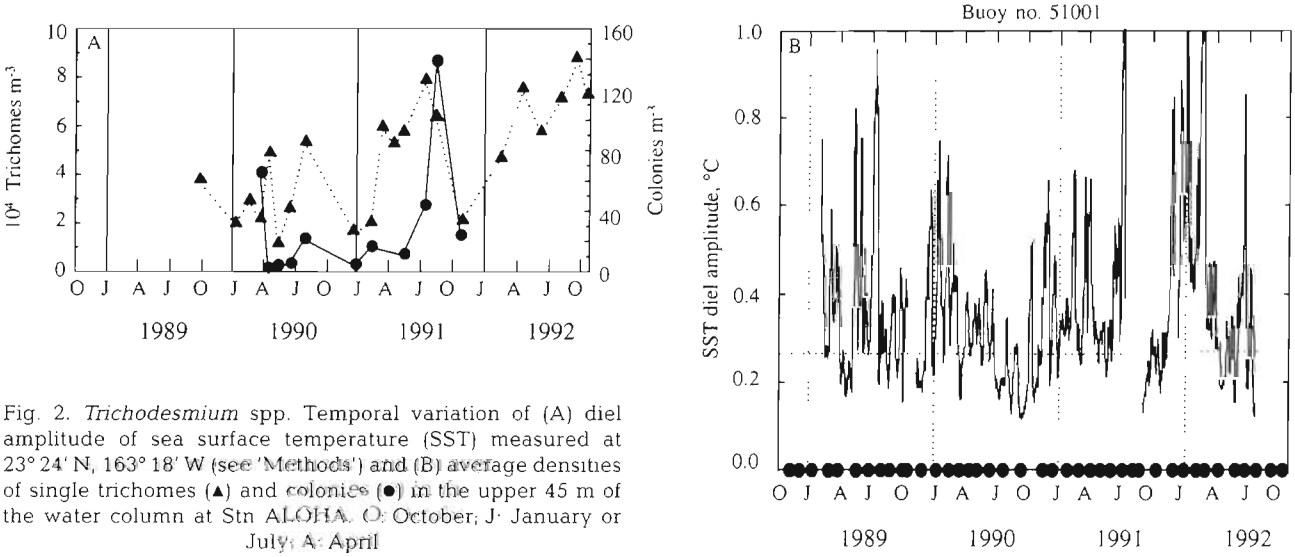

Fig. 2. *Trichodesmium* spp. Temporal variation of (A) diel amplitude of sea surface temperature (SST) measured at 23° 24' N, 163° 18' W (see 'Methods') and (B) average densities of single trichomes (▲) and colonies (●) in the upper 45 m of the water column at Stn ALOHA. O: October; J: January or July; A: April

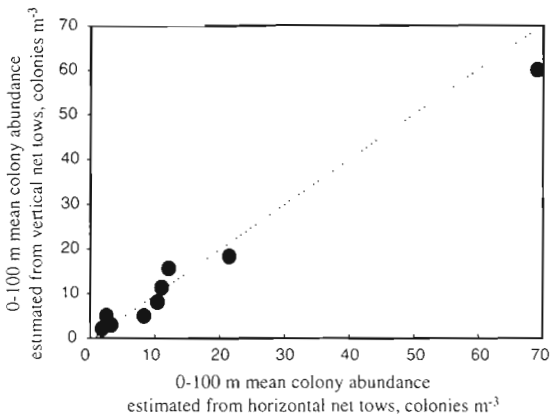

Fig. 3. *Trichodesmium* spp. Comparison between the mean abundance colonies over the upper 100 m of the water column estimated from 0 to 100 m vertical net tows and from horizontal net tows performed at discrete depths

relation between SST amplitude and trichome abundance (Kendall rank correlation $p > 0.1$ for all running averages). Colonies appear to increase in abundance only after extended periods of water column stratification (Fig. 2A).

Single trichome concentrations follow a seasonal cycle with low densities ($<4.0 \times 10^4$ trichomes m^{-3}) observed during winter and spring months. Trichome abundance also displays an interannual variability with significantly higher values of single trichomes in the water column during 1991 and 1992 (Wilcoxon 2 sample test $p < 0.05$; Fig. 2).

During 1991 and 1992, *Trichodesmium contortum*, a species not described previously for the central North Pacific Ocean and characterized by the screw-like shape of its trichomes, appeared sporadically in the net samples. Although *T. contortum* was never abundant

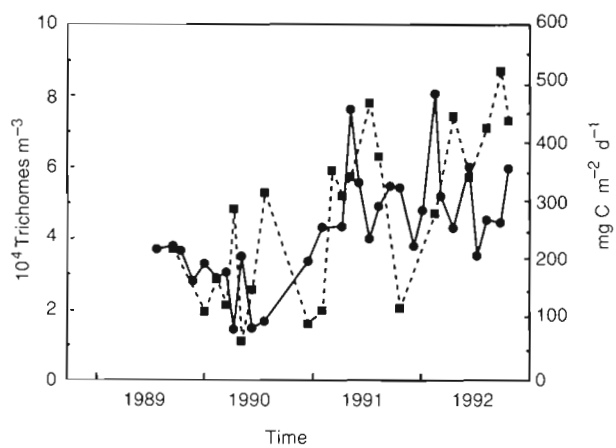

Fig. 4. *Trichodesmium* spp. Temporal variation of photosynthetic carbon assimilation (●) integrated over the upper 45 m depth and average density of single trichomes (■) over the same depth range

Table 1. *Trichodesmium* spp. Elemental composition and chlorophyll *a* (chl *a*) content of single trichomes and colonies [mean (±SD)]. PC: particulate carbon; PN: particulate nitrogen; PP: particulate phosphorus

Parameter	Single		Colonies[a]
	May 90	Oct 91	
Chl *a* (ng filament^{-1})	0.27	0.26	0.32 (0.034)
PC (ng filament^{-1})	50.0	51.6	52.7 (1.8)
PN (ng filament^{-1})	9.2	9.8	9.6 (0.4)
PP (ng filament^{-1})	0.40	0.48	0.51 (0.07)
PC:chl *a* (w:w)	187	199	163 (29.2)
PC:PN (mol:mol)	6.34	6.14	6.4 (0.35)
PN:PP (mol:mol)	52	45	42 (6.15)

[a]Assumes colonies composed of 182 filaments

in our samples [<1% of the total *Trichodesmium* biomass (*T. thiebautii* and *T. erytheaum* are the dominant species)], it was always present at one or several depths in all cruises between April 1991 and October 1992.

An increase in primary production in the upper 45 m of the water column was also observed during 1991 and 1992 (Karl et al. 1995; Fig. 4) suggesting that the abundance of trichomes and the amount of carbon production may be controlled by the same factors. However, short term increases in primary production appear to be correlated with decreases in the abundance of trichomes (Fig. 4).

The mean elemental composition of colonies as well as single trichomes does not vary significantly between the 2 morphologies; only the carbon to chlorophyll ratio between single trichomes and colonies varies (Table 1). The C:N ratio was remarkably similar when comparing results from samples collected in May 1990 and October 1991. These results contrast with the observed change in the elemental composition of the bulk particulate matter in the upper 45 m of the water column between 1989 and 1992 (Table 2). Based on

Table 2. Elemental ratio of 0 to 45 m depth integrated particulate matter at Stn ALOHA and comparison of the median elemental ratio with the theoretical Redfield ratio of 106C:16N:1P

Elemental ratio	Period	Mean (mol:mol)	Median (mol:mol)	Significance level[a]
PC:PN	1989–90	9.06	7.08	$0.05 < p < 0.1$
	1991–92	7.22	6.86	$p > 0.1$
PC:PP	1989–90	114.1	113.7	$p > 0.1$
	1991–92	132.9	132.0	$p < 0.001$
PN:PP	1989–90	14.1	14.6	$p > 0.1$
	1991–92	19.3	18.2	$p < 0.05$

[a]Based on Wilcoxon's ranks test

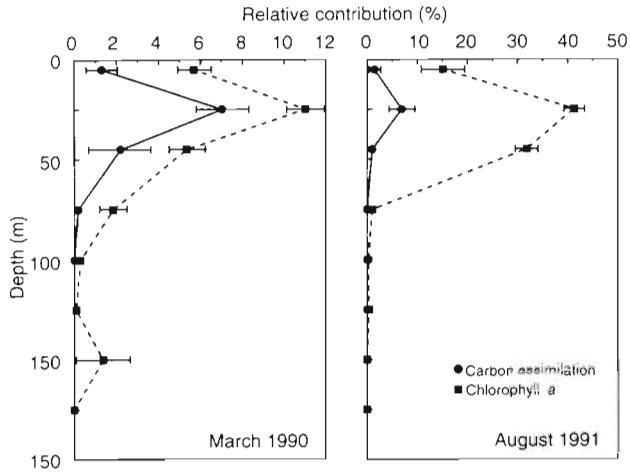

Fig. 5. *Trichodesmium* spp. Depth distribution of the contribution of *Trichodesmium* spp., as percentage, to total photosynthetic carbon assimilation (●) and total chl *a* (■)

Wilcoxon's signed rank test analyses, the phosphorus content in the suspended particulate matter for the period 1991–1992 is on average significantly lower than the Redfield ratio (Redfield et al. 1963).

Even though chl *a* contributed by *Trichodesmium* may be as high as 40% of the total chl *a* measured at a discrete depth (Fig. 5), integrated values from 0 to 45 m account for no more than 28% ($\bar{x}$ = 18%, s = 8%, n = 22). Carbon assimilation contribution is always lower than the chl *a* contribution (Fig. 5) suggesting that the carbon assimilation efficiency of *Trichodesmium* is lower than the assimilation efficiency of the phytoplankton community as a whole.

DISCUSSION

The only report available on the spatial distribution of single filaments of *Trichodesmium* (trichomes) in the water column derives from a study in the Central and North Pacific Ocean. On a transect from 50° N to 10° S along 155° W, Marumo & Asaoka (1974b) found large concentrations of filaments (>5 × 10^4 trichomes m^{-3}) restricted to the upper 50 m of the subtropical gyre (from 15° N to 29° N). These concentrations are higher, but not significantly different from the average abundance of single trichomes found in the upper water column of Stn ALOHA ($\bar{x}$ = 4.6 × 10^4 trichomes m^{-3}, s = 2.3 × 10^4 trichomes m^{-3}, n = 22). By comparing these concentrations with the abundance of *Trichodesmium* cells in the colony morphology found at Stn ALOHA, we conclude that colonies comprise a minor fraction (12%) of the *Trichodesmium* population in our study.

Due to the methodologies used to collect single trichomes and colonies in the present study, the possibility of overestimating single trichome and underestimating colony densities as a result of the mechanical disruption of colonies exists. However, the slope of the linear regression between integrated colony concentration calculated from vertical net tows and from samples collected at discrete depths (horizontal net tows) was not different from 1.0 (95% confidence limits = 0.84 and 1.06, R^2 = 0.85, n = 9; Fig. 3). Because, on average, each horizontal net tow filtered 43 m^3 and vertical net tows only filtered 16 m^3 this result suggests that the percentage of colonies disrupted is not a function of the volume filtered by the net. Given the average abundance of colonies estimated for the upper 50 m of the water column ($\bar{x}$ = 31 colonies m^{-3}), the complete disruption of *Trichodesmium* bundles would account for less than 14% of the estimated average abundance of single trichomes. Furthermore, because colonies are probably subjected to a higher stress during a net tow than during a rosette water sampling procedure, we believe that our calculations of single trichome densities are not significantly affected by colony disruption.

Carpenter & Romans (1991) calculated from historical data that *Trichodesmium* colonies contribute 60% of the total chl *a* measured in small sample volumes (200 to 300 ml) collected in the upper water column of the western North Atlantic Ocean. This high contribution indicates that most of the *Trichodesmium* biomass in the western North Atlantic Ocean must be in the colonial morphology. Free trichomes were not enumerated in that study. A marked difference in morphological composition and in the difference of the estimated contribution of *Trichodesmium* to organic matter (Table 3) suggests that there may be significant ecological differences between these 2 marine environments. This conclusion precludes the possibility of freely extrapolating results from one ocean basin to another.

Although there is no significant correlation between calm weather periods, as distinguished by the diel

Table 3. *Trichodesmium* spp. Temporal variation in the contribution of single trichomes to total suspended particulate carbon (PC), particulate nitrogen (PN), particulate phosphorus (PP), and chlorophyll *a* (Chl *a*) in the upper 45 m of the euphotic zone at Stn ALOHA

Parameter	1989–1992 (%)	1989–1990 (%)	1991–1992 (%)
PC	9.2 ± 3.4[a]	5.8 ± 2.7	11.3 ± 3.3
PN	10.8 ± 4.5	7.2 ± 2.9	13.3 ± 3.7
PP	4.6 ± 2.4	2.2 ± 1.1	5.3 ± 1.9
Chl *a*	13.4 ± 8.1	8.1 ± 6.4	17.1 ± 7.0

[a]Mean ± 1 SD of the mean

amplitude of the SST at 23° 24' N, 162° 18' W, and the abundance of single trichomes in the upper 45 m of the euphotic zone at Stn ALOHA, low *Trichodesmium* densities appear consistently during deep mixing events and winter months. Nevertheless, low concentrations observed during May to June 1990 and October 1991 do not appear to be the result of deep mixing events. These observations suggest that environmental factors other than wind driven events and sea surface temperatures, perhaps cloud cover, solar irradiance and grazing pressure, may play an important role in controlling *Trichodesmium* distribution and abundance on short time scales (days).

On larger time scales, the interannual variability in *Trichodesmium* abundance observed in the upper 45 m of the water column during 1991 to 1992 may be the result of a decrease in the frequency of deep mixing events (Karl et al. 1995). Extended periods with a shallow mixed-layer will benefit phytoplankton such as *Trichodesmium* that have the capacity to regulate their buoyancy (Fogg 1982, Komopka 1984, Karl et al. 1992).

The elemental composition of *Trichodesmium* is surprisingly constant when comparing single trichomes and colonies (Table 1). The PC:PN molar ratio (6.3:1) is close to the predicted Redfield ratio (6.6:1) but the PN:PP (45:1) is 3 times greater than Redfield stoichiometry (16:1). These results are consistent with observations reported in previous studies (Mague et al. 1977, McCarthy & Carpenter 1979, Lewis et al. 1988, Karl et al. 1992, 1995). A low phosphorus cell quota appears as a characteristic of *Trichodesmium* cells whether in free trichome or colony morphology. Furthermore, the chemical analysis of actively photosynthetic colonies collected during a bloom event in the vicinity of Stn ALOHA yielded a PN:PP ratio of 125:1 (Karl et al. 1992), suggesting a large flexibility in the cell quota phosphorus in *Trichodesmium*.

The contribution to the integrated photosynthetic carbon assimilation by *Trichodesmium* during March 1990 and May 1991 in the upper 45 m of the water column at Stn ALOHA was estimated to be 3.9 and 4.3% of the total production, respectively (Fig. 5). Although the primary production below 45 m accounted for 65 and 43% of the 0 to 200 m integrated production, the contribution by *Trichodesmium* was negligible below 75 m. Based on these results, the estimated contribution of *Trichodesmium* to total inorganic carbon assimilation was approximately 1.7% during March 1990 and 2.5% during August 1991

The fraction of autotrophic production attributed to *Trichodesmium* does not appear to be important unless we consider this contribution in terms of new production. Based on the downward flux of PN measured at Stn ALOHA and assuming that photoautotrophic production of particulate matter is close to C:N Redfield ratio, the estimates of particulate exported production at 150 m [downward particulate flux × (total production)$^{-1}$] range from 0.02 to 0.12 between 1989 and 1992 ($\bar{x}$ = 0.06, s = 0.02, n = 39).

Field incubations (Mague et al. 1977), as well as the nitrogen isotopic signature of *Trichodesmium* ($\delta^{15}N$ = −1.7 to +0.5 ppm; Wada & Hattori 1976) indicate that nitrogen fixation accounts for virtually all cellular nitrogen required for this organism to grow. Because the N:C ratio observed for *Trichodesmium* is not significantly different from the Redfield ratio, we can use carbon assimilation as an estimate of nitrogen fixation. If the values of carbon assimilated by *Trichodesmium* measured during March 1990 and August 1991 are representative of its contribution to production at Stn ALOHA, then *Trichodesmium* accounts, on average, for 27% of new production (21% during 1989 to 1990 and 35% during 1991 to 1992). However, this estimate should be viewed as a lower boundary value because it does not take into account either bloom events (Karl et al. 1992) or the potential release of NH^+_4 and dissolved organic nitrogen (DON) by *Trichodesmium* during nitrogen fixation (see below).

The highest carbon assimilation observed in the first 4 yr of the HOT program occurred during August 1989. Although an increase in the abundance of *Trichodesmium* was observed at the time of this primary productivity experiment, a large bloom did occur 3 d later at Stn ALOHA (Karl et al. 1992). This observation suggests that trichomes were already abundant at the time of the incubations. However, there is no evidence of an increase in phytoplankton biomass when comparing the concentration of chl *a* measured from the primary productivity cast with values obtained in either previous or subsequent months (Fig. 6A). The only strong evidence suggesting that a change occurred in the water column was found in the carbon assimilation efficiency of the phytoplankton (P^B) and the C:N ratio of particulate matter. The carbon assimilation efficiency increased by a factor of 2 in the upper 50 m the water column (Fig. 6B) while the C:N ratio decreased from 7.3:1 to 6.3:1 (mol:mol; Fig. 6C). Assuming that the Redfield ratio for phytoplankton is 6.6:1, the shift in the particulate elemental ratio suggests that phytoplankton were not nitrogen limited during August 1989.

Trichodesmium carbon assimilation number (P^B) reported in the literature and calculated for Stn ALOHA over the period of this study are relatively low, ranging from 0.17 to 3.8 g C (g chl *a*)$^{-1}$ h^{-1} (Carpenter & Price 1977, Mague et al. 1977, Lewis et al. 1988, Karl et al. 1992; Fig. 7). Hence, the increase in P^B observed in August 1989 and during 1991–1992 cannot be explained by an increase of the contribution of *Trichodesmium* to the total production in the euphotic zone.

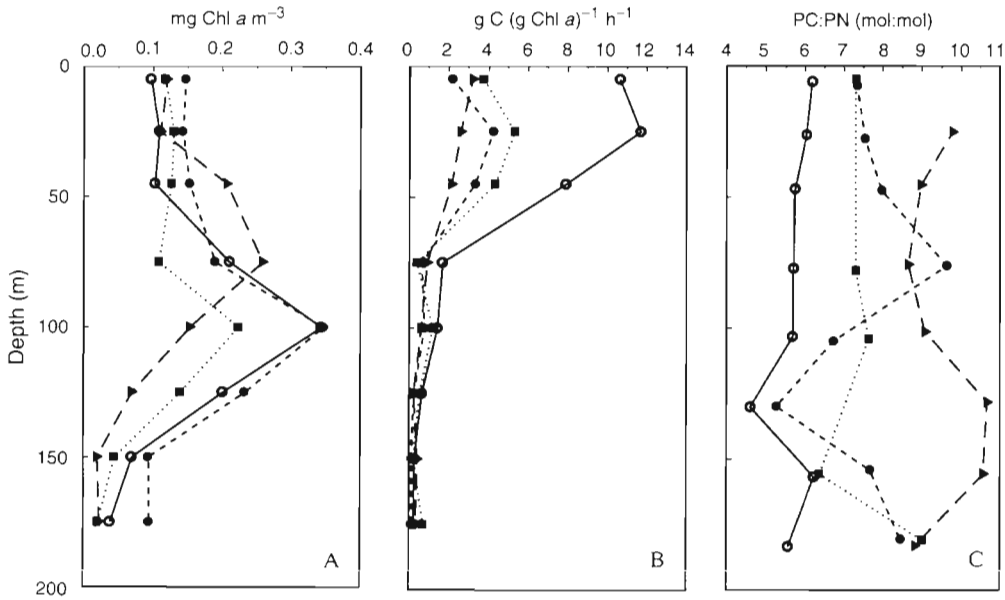

Fig. 6. Depth profiles of (A) chl *a* concentrations, (B) phytoplankton assimilation efficiency, and (C) molar ratio of carbon to nitrogen in the suspended particulate matter measured in samples collected at Stn ALOHA between July and November 1989. (●) July; (○) August; (■) September; (▲) November samples

However, 2 lines of evidence suggest the existence of a synergistic effect between *Trichodesmium* and other algae present in the water column. The first observation is derived from the primary productivity experiments performed during March 1990 and August 1991 (Fig. 7). In both cases the incubations at 25 and 45 m showed that the P^B of samples containing 1 colony was higher than the calculated P^B based on results from the independent incubation of colonies and phytoplankton. This effect was not evident at 5 m depth and may have been inhibited by high light conditions (Li et al. 1980, Lewis et al. 1988).

If we consider the supply of nitrogen to be limiting the algal production in the upper euphotic zone it is possible to speculate that a release of NH_4^+ and dissolved organic matter rich in DON during nitrogen fixation (Prufert-Bebout et al. 1993, Glibert & Bronk 1994) will enhance the production of phytoplankton other than *Trichodesmium*. Under high irradiance (the 5 m depth sample) the production of oxygen due to photosynthesis could be inhibiting nitrogenase activity thus suppressing nitrogen fixation. *Trichodesmium* colonies have high respiration rates (Kana 1992), and the light intensity of the compensation point for oxygen evolution in these colonies is approximately 300 μE m^{-2} s^{-1} (or 13 E m^{-2} d^{-1}) corresponding to the irradiance measured at 30 m depth at Stn ALOHA. One of the metabolic benefits of a high respiration rate is the protection of nitrogenase activity by reducing the intracellular oxygen tension during daylight hours. From this perspective, trichomes located near the 300 μE m^{-2} s^{-1} isolume may have higher nitrogenase activity than those located closer to the sea surface.

Nevertheless, we need to be careful with the interpretation of historical values of P^B given for *Trichodesmium*, and with the results of the experiment discussed above. Assimilation numbers in *Trichodesmium* have been calculated only by using colonial morphologies where trichomes are subject to self shading effects. In the samples collected during this study, only chl *a* concentration per unit biomass (PC) increases in colonies when compared to single trichomes (Table 1). Although the increase in this ratio is not significant due to the variability associated with measurement in colonies, it suggests that some degree of photoadapta-

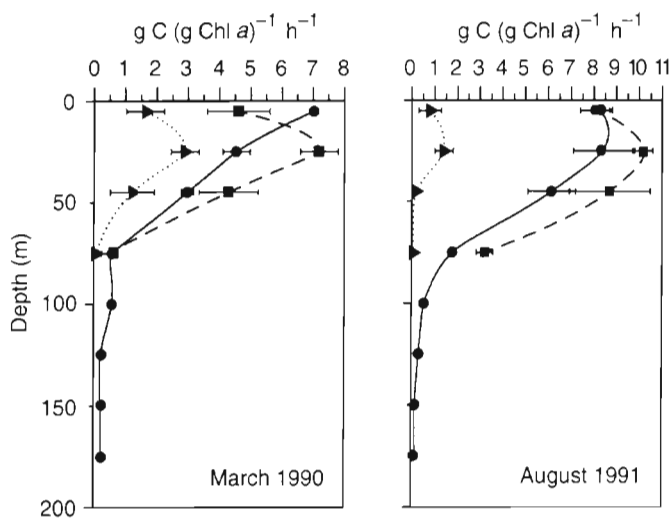

Fig. 7. *Trichodesmium* spp. Depth distribution of assimilation efficiency measured simultaneously in colonies (▲), natural phytoplankton assemblages (●), and natural phytoplankton assemblages amended with colonies (■). Error bars represent standard deviations

tion may occur in colonies as a result of self shading. This observation is also consistent with the decrease in PC:chl *a* observed during bloom conditions (Karl et al. 1992). However, the relative decrease in PC:chl *a* in colonies found under non-bloom conditions during this study suggests that photoadaptation between different morphologies is not strong and may account for no more than a 20% increase of P^B between colonies and single trichomes (Table 1).

The possibility that handling the colonies in the preparation of primary production experiments may have disrupted microenvironments (Carpenter & Price 1976, Martinez et al. 1983) and damaged trichomes cannot be excluded (but see Carpenter et al. 1987 and Scranton et al. 1987). Although colonies inspected under a microscope before the inoculations did not appear broken, 20% of the incubation bottles at the end of the incubation period had some single trichomes. If damage occurred in the colonies the low P^B value calculated for colonies must be considered an artifact of the experimental manipulations.

A second line of evidence suggesting that *Trichodesmium* is a source of nitrogen for other phytoplankters comes from the observed shift in elemental ratio of the particulate matter and its correlation with the abundance of trichomes in the upper 45 m of the water column (Fig. 8). Sixty percent of the variability in PN:PP may be attributed to variations in the abundance of *Trichodesmium*. Nevertheless, an increase in the percentage contribution of *Trichodesmium* biomass to the total suspended particulate matter is not sufficient to explain the change in the elemental ratio of the suspended particulate matter (Table 2).

By knowing the approximate contribution of carbon, nitrogen, and phosphorus in *Trichodesmium* (Table 3), it is possible to calculate the elemental composition of

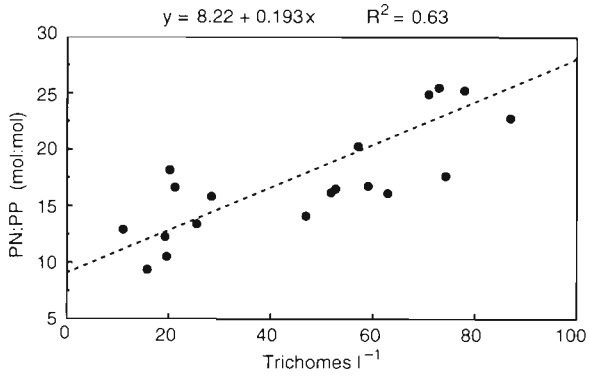

Fig. 8. *Trichodesmium* spp. Molar ratio of 0 to 45 m depth integrated nitrogen (PN) to phosphorus (PP) concentration in suspended matter plotted against the average concentration of single trichomes over the same depth range. A model II geometric mean linear regression was used to model the relation between both parameters

Table 4. Temporal variation in the elemental ratio of suspended particulate matter after removing the contribution of single trichomes in the upper 45 m of the euphotic zone at Stn ALOHA

Elemental ratio	1989–1992 (mol:mol)	1989–1990 (mol:mol)	1991–1992 (mol:mol)
PC:PN	7.7	8.0	7.3
PN:PP	15.3	13.5	16.6

the remaining bulk particulate matter. This residual PN:PP of the particulate organic matter in the upper 45 m of the water column, excluding *Trichodesmium*, varies from 13.5:1 (mol:mol) in 1989–1990 to 16.6:1 (mol:mol) in 1991–1992 (Table 4) suggesting that nitrogen is not a limiting nutrient during the latter period of the present study.

Two different processes related to the presence of *Trichodesmium* may be taking place in the euphotic zone. The first, discussed above, is the release of NH_4^+ and DON during the process of nitrogen fixation. The second process is the release of other essential nutrients during the senescence of trichomes. Evidence in the literature indicates that *Trichodesmium* degrades very fast (hours to days) upon reaching senescence (Van Baalen & Brown 1969, Borstad & Borstad 1984). If this is the case at Stn ALOHA, a decrease in the concentration of trichomes between cruises could result in the release of nutrients, such as phosphorous, to other phytoplankton taxa. This interpretation is consistent with the observed trend of photosynthetic carbon assimilation and *Trichodesmium* densities at Stn ALOHA. The increase in abundance of trichomes in the upper water column during 1991 to 1992 coincides with an increase of photosynthetic carbon assimilation although, on smaller temporal scales (weeks to month), primary production appears to be inversely correlated to *Trichodesmium* abundances (Fig. 4). The increase in carbon assimilation rates, following a decrease in trichome abundance between cruises, may indicate that the release of nutrients as a result of *Trichodesmium* senescence is an important ecological process, even under non-bloom conditions.

Karl et al. (1995) have suggested that the biogeochemical shift observed between 1989 to 1990 and 1991 to 1992 may be a result of an increase of the stratification in the water column during an El Niño-Southern Oscillation (ENSO) event. If the coupling between *Trichodesmium* abundance, water column stratification, and ENSO periods proves to be correct, we should expect to observe a relaxation of nitrogen deficiency of the pelagic ecosystem of the North Pacific subtropical gyre during ENSO events. This relaxation would result from the combined effect of the reduction

of nutrient inputs due to deep mixing events and the increase of nitrogen fixation by *Trichodesmium* and other diazotrophs.

In conclusion, the observed change in PN:PP in the upper 45 m of the water column at Stn ALOHA appears to be the result of a decrease in the frequency of deep mixing events. Because of the capacity to regulate its buoyancy to fix N_2, *Trichodesmium* may play a significant role in the input of nutrients when the euphotic zone water column is stratified for extended periods of time. Furthermore, an over production of reduced nitrogen and the concomitant extracellular release of ammonium and DON by *Trichodesmium* may explain the relative enrichment in PN observed at Stn ALOHA during 1991 and 1992.

Perhaps because the focus of research on oceanic photoautotrophic production was historically influenced by problems related to the management of fisheries (Ryther 1969), the contribution of nitrogen fixation to total production estimated for the North Pacific subtropical gyre may have been considered of little importance. However, the contemporaneous concepts of new production and exported biogenic matter from the euphotic zone have become central in the analysis of biogeochemical cycles in the ocean during the past decade (Longhurst 1991). This is the result of our interest and necessity to estimate the role of the oceans in global carbon cycles. In this context, results obtained during the first 4 yr of the HOT-series program suggest that the contribution of nitrogen fixation by *Trichodesmium* in the North Pacific subtropical gyre may provide a significant fraction of the nitrogen supporting new production.

Acknowledgements. We thank the captains, crews and scientists participating in the HOT program for assistance in the collection and analysis of samples. We also thank Roger Lukas and the HOT-WOCE team for the generation of hydrographic data, the National Oceanic and Atmospheric Administration-National Data Buoy Center (NOAA-NDBC) for access to the ocean data recorded by buoy #51001, and E. J. Carpenter and 2 anonymous reviewer for helpful comments and suggestions. The present study was supported by National Science Foundation grants OCE 88-00329, OCE 90-16090, and OCE 93-01368 (awarded to D.K.), and OCE 87-17195 and OCE 93-03094 (awarded to R. Lukas). SOEST publication no. 3937.

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This article was presented by B. and E. Sherr (Senior Editorial Advisors), Corvallis, Oregon, USA

Manuscript first received: March 8, 1995
Revised version accepted: September 27, 1995