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Spatial and seasonal variations of Cyanobacteria and their nitrogen fixation rates in Sanya Bay, South China Sea

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SUMMARY: The nitrogen fixation rates of planktonic and intertidal benthic cyanobacteria were investigated in Sanya Bay from 2003 to 2005. *Trichodesmium thiebautii* was the dominant species of planktonic cyanobacteria during our study. Significant seasonal and spatial variations in *Trichodesmium* spp. abundance were observed (P<0.01). The highest *Trichodesmium* concentrations occurred during intermonsoon periods and in the outer region of Sanya Bay (Outer Bay stations). At fixed station S03 the abundance of *T. thiebautii* ranged from 1.14×10^3 to 2060×10^3 trichomes m^{-2}, with an annual mean of 273×10^3 trichomes m^{-2}. The average nitrogen fixation rate per colony of *T. thiebautii* was 0.27 nmol N h^{-1} $colony^{-1}$ and it did not show any obvious seasonal variations. Nitrogen fixation by planktonic cyanobacteria was highest in the Outer Bay stations, where the estimated amount of new nitrogen introduced by *Trichodesmium* contributed 0.03 to 1.63% of the total primary production and up to 11.64% of the new production. Statistical results showed that significant seasonal and spatial variations of nitrogen fixation rates were found among the intertidal communities. The main benthic nitrogen-fixing cyanobacteria were identified as members of the genera *Anabaena*, *Calothrix*, *Lyngbya*, *Nostoc* and *Oscillatoria.* The highest nitrogen fixation rate was found in microbial mats and the lowest in reefs and rocky sediments. All the benthic communities studied presented their highest nitrogen fixation activity in summer, with an average nitrogen fixation rate of 33.31 µmol N h^{-1} m^{-2}, whereas the lowest nitrogen activity was detected in winter, with an average nitrogen fixation rate of 5.66 µmol N h^{-1} m^{-2}. A Pearson correlation analysis indicated that the nitrogen fixation rate of three types of intertidal communities was significantly positively correlated to seawater temperature (P<0.05), whereas only the nitrogen fixation rate of the reefs and rock communities was significantly negatively correlated to seawater salinity (P<0.05).

Keywords: *Trichodesmium*, phytoplankton, nitrogen fixation, cyanobacteria, Sanya Bay.

RESUMEN: VARIACIÓN ESPACIAL Y ESTACIONAL DE CIANOBACTERIAS Y SUS TASAS DE FIJACIÓN DE NITRÓGENO EN LA BAHÍA DE SANYA EN EL SUR DEL MAR DE CHINA. – Las tasas de fijación de nitrógeno de cianobacterias intermareales y bentónicas fueron investigadas en la Bahía de Sanya, desde 2003 a 2005. *Trichodesmium thiebautii* era la especie dominante de las cianobacterias planctónicas durante nuestra investigación. Se observaron variaciones espaciales y estacionales significativas (P<0.01) en la abundancia de *Trichodesmium* spp. La concentración más elevada de *Trichodesmium* se observó durante los períodos de intermonzón y en la región exterior de la Bahía de Sanya (estaciones fuera de la Bahía). En la estación fija S03 la abundancia de *T. thiebautii* variaba desde 1.14×10^3 a 2060×10^3 tricomas m^{-2}, con una media anual de 273×10^3 tricomas m^{-2}. El promedio de la tasa de fijación de nitrógeno por colonia de *T. thiebautii* era de 0.27 nmol N h^{-1} colonia y no mostraba una clara variación estacional. La fijación de nitrógeno por las cianobacterias planctónicas era superior en las estaciones de fuera de la Bahía, donde la cantidad estimada de nitrógeno nuevo introducido por *Trichodesmium* contribuía del 0.03 al 1.63% del total de la producción primaria y hasta el 11.64% de la producción nueva. Estadísticamente los resultados mostraban que las variaciones espaciales y estacionales significativas de las tasas de fijación de nitrógeno fueron encontradas entre las comunidades intermareales. Las principales cianobacterias bentónicas fijadoras de nitrógeno fueron identificadas como miembros de los géneros *Anabaena*, *Calothrix*, *Lyngbya*, *Nostoc* y *Oscillatoria*. La tasa de fijación de nitrógeno más alta fue encontrada en los tapetes microbianos y las más bajas en los arrecifes y sedimentos rocosos. Todas las comunidades bentónicas estudiadas presentaban la mayor actividad de fijación de nitrógeno en verano, con un promedio de tasas de fijación de 33.31 µmol N h^{-1} m^{-2}, mientras que la menor actividad de fijación de nitrógeno fue detectada en invierno, con un promedio de 5.66 µm

N h^{-1} m^{-2}. Análisis de correlación (Pearson) indicaban que las tasas de fijación de nitrógeno en los tres tipos de comunidades intermareales estaban significativamente correlacionados con la temperatura del agua (P<0.05). Mientras que la tasa de fijación de nitrógeno de las comunidades de los arrecifes y sedimentos rocosos estaban correlacionadas significativamente con la salinidad del agua de mar (P<0.05).

Palabras clave: *Trichodesmium*, fitoplancton, fijación de nitrógeno, cianobacteria Bahía de Sanya.

INTRODUCTION

Cyanobacteria are important contributors to open water and benthic oceanic primary production through photosynthesis and nitrogen (N) fixation (Hoffmann, 1999; Lugomela *et al.,* 2002; Karl *et al.,* 2002; Omoregie *et al.,* 2004). *Trichodesmium*, equipped with buoyancy-regulating gas vesicles and nitrogen fixation enzymes, is considered to be well adapted to tropical and subtropical oligotrophic oceans (Capone *et al.,* 1997), where it contributes a major fraction of new nitrogen to oligotrophic surface waters (Karl *et al.,* 1997; Lugomela *et al.,* 2002). In near-shore environments, there is usually a large diversity of benthic cyanobacteria (Zehr *et al.,* 1995; Arnaud *et al.,* 2003). They are found in many locations, such as within coral skeletons, in association with algae in soft sediments between colonies, on rock, and as epiphytes on seagrasses and other macrophytes (Dong *et al.,* 2002b; Dong *et al.,* 2006). N fixation represents a significant amount of 'new nitrogen' in intertidal and marine ecosystems (Capone and Carpenter, 1982; Zehr *et al.,* 1995; Capone *et al.,* 1997; Karl *et al.,* 1997; Roubaud *et al.,* 2001; Chen *et al.,* 2003). For example, nitrogen fixation by bacteria associated with leaves, roots and rhizomes was the main source of nitrogen in the Gulf of Elat (Red Sea) (Pereg-Gerk *et al.,* 2002), and in seagrass beds in the Atlantic coast of the US (Smith and Hayasaka, 1982). According to some estimates, up to 50% of plants' nitrogen requirements may come from nitrogen fixation within the rhizosphere sediments (Patriquin, 1972; Capone and Taylor, 1980; O'Donohue *et al.,* 1991).

Sanya Bay, a typical tropical bay, lies in the southernmost part (109°20'-109°30'E, 18°11'-18°18'N) of Hainan Island in China (Fig. 1), with a water surface area of 120 km^2 and an average depth of 16 m (Huang *et al.,* 2003). The islands of Dongmao, Ximao and Luhuitou are located at the mouth of the bay. Sanya Bay is warm and oligotrophic, and N is the main nutrient limiting primary production, especially during summer and autumn (Dong *et al.,* 2002a; Huang, 2003). Benthic cyanobacteria presented high biodiversity in Sanya Bay and were often abundant in rocks and coral reefs (Dong *et al.,* 2006). Coastal coral reefs, which account for 30% of the total coastline, are concentrated in the vicinity of Luhuitou and the islands to the east and west of the mouth of the bay (Huang *et al.,* 2003), and represent the main benthic habitat in the bay. Epiphytic cyanobacteria such as *Lyngbya* spp., which usually grow loosely attached to seagrass and macroalgae, are the main cyanobacteria in seagrass and macroalgal communities, and are found in estuaries and shallow open shelves, and adjacent to coral reefs off the coast of Sanya Bay. Microbial mats, consisting of dense, complex assemblages of cyanobacteria, bacteria and algae (Stal, 1995; Omoregie *et al.,* 2004), are present on shallow coral and sandy coasts and occupy an annual mean area of about 0.9 km^2 in the bay.

In this paper we determined the abundance of nitrogen-fixing cyanobacteria and analyzed nitrogen fixation rates in the water column and intertidal zone of Sanya Bay, in order to provide information on their spatio-temporal variability and on the contribution of nitrogen fixation to primary production in the Sanya Bay ecosystem. Nitrogen fixation data for Sanya Bay are important for understanding the biogeochemical role of cyanobacteria in tropical ecosystems.

MATERIAL AND METHODS

Between January 2003 and December 2005, we sampled different populations in the natural ecosystem of Sanya Bay. The study included two components: (1) results of monthly cruise surveys in fixed station S03 and four cruise surveys of stations S01 to S11, which described the spatial and temporal variations of nitrogen fixation and planktonic cyanobacteria distribution in the bay waters, and (2) sample collection and benthic nitrogen fixation assays in the intertidal zone to show seasonal variations of nitrogen fixation rates. The monthly cruises to station S03 were conducted from January 2 to December 11 2003 (Table 2). The S01 to S011 cruises were conducted in January 12-13, April 13-14, August 18-19 and October 22-23, 2004. The sampling stations

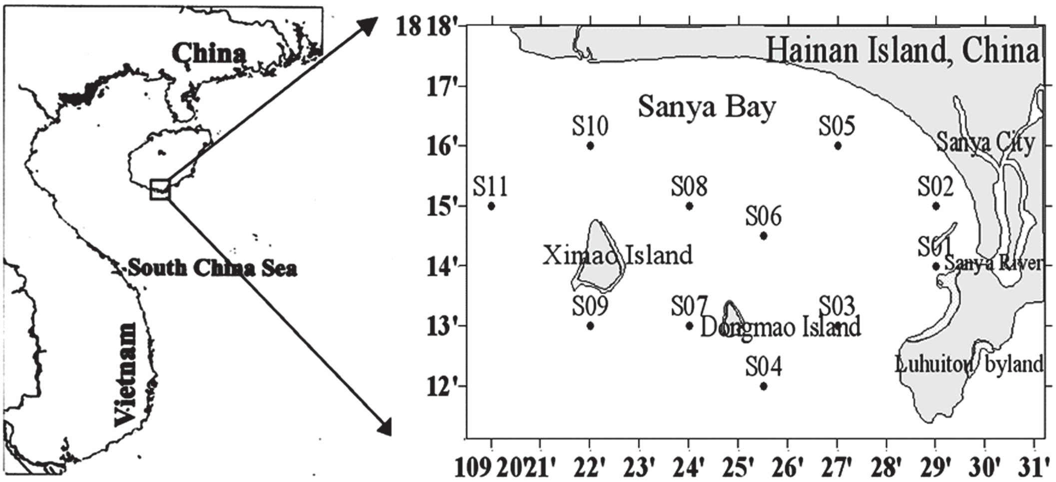

FIG. 1. – Location of sampling sites and research stations in Sanya Bay.

during these cruises were selected to represent the three environments of Sanya Bay: the Coastal Bay (S01, S02 and S05), Mid Bay (S03, S06 and S08) and Outer Bay (S04, S07, S09, S10 and S11) (Fig. 1). The samples collected from the intertidal zone included three main community types: reefs and rocky sediments (RR), seagrass and macroalgae ecosystem (SM) and microbial mats (MM). The samples were grouped according to collection dates: "spring" (April 15, 2004 and April 10, 2005), "summer" (July 6, 2005 and August 15, 2005), "autumn" (September 8, 2004, September 13 and 14, 2004, and September 22, 2005) and "winter" (January, 10, 2004, December 27, 2004 and December 7, 2005). Samples were collected early in the morning or late in the afternoon, when the tide was low.

Surface and bottom water temperature and salinity were determined in situ using a Quanta Water Quality System. Water samples were collected with 5 L Niskin bottles attached to a nylon cable. The mean of surface and bottom values was used to characterize the water column. Two replicates of 1.5 L samples from the depths mentioned above were filtered immediately through pre-cleaned 0.45 µm pore-size cellulose filters for nutrient analyses. The samples were preserved deep frozen in the dark before analyzing the nitrate and silicate with a SKALAR autoanalyzer (Skalar Analytical B.V. SanPlus, Holland). Ammonium and phosphorus were analyzed with oxidation methods using hypobromite and molybdophosphoric blue with a UV1601 spectrophotometer (SHIMADZU Corporation). The primary production (PP) was measured using the ^{14}C method as described by Tan *et al.,* (2004). The samples were collected before noon at 100%, 50%, 30% and 1% of the surface irradiance and pre-filtered through a 200 µm screen to remove larger zooplankton. Duplicate 50 ml samples were inoculated with 1 or 2 µCi ^{14}C-labeled sodium bicarbonate and incubated for about 4 hours under direct sunlight, using neutral density screens to simulate the light intensity from which the samples were collected. The temperature in the on-deck incubators was regulated by flowing surface seawater. Dark bottles were incubated simultaneously under the same conditions. The incubations with ^{14}C were terminated by filtering the samples through 0.45 mm membrane filters using a filtration vacuum <100 mm Hg. To remove inorganic ^{14}C the filters were placed on the bottom of a glass scintillation vial that contained HCl. The filters with organic ^{14}C were kept frozen at –20ºC. Before analysis, the membrane filters were digested with HCOOH for about 2.5 h. The samples were counted with a 2000 CA/LL Liquid Scintillation Analyzer, and primary production was calculated according to Parsons *et al.,* (1984).

Water samples for planktonic cyanobacteria enumeration and nitrogen fixation experiments were collected at the 11 sampling stations. A phytoplankton net (0.5 m diameter, length 208 cm, 20 µm mesh) with a flow meter was towed vertically from 1.5 m above the seabed to the surface. Phytoplankton samples were rinsed and preserved in 5% formalin for further identification of species and counting in the laboratory. Microbial mat samples were collected by the method described in Bebout *et al.,* (1987), at low tide (no overlying water). Typical assemblages of intact dead coral, shells, reefs, etc. were collected from coral reef communities. Samples from the seagrass beds were taken with a scoop at low tide. The samples, including macroalgae and seagrass leaves

TABLE 1. – Statistical data (means± Std. deviation) of some environmental factors of Sanya Bay from 2003 to 2005.

Season	Temperature (ºC)	Salinity	Average (μmol l-1) NO_3^-	NO_2^-	NH_4^+	PO_4^{3-}	SiO_3^{2-}
Winter	23.49±0.16	34.41±0.16	1.01±0.27	0.06±0.07	0.92±0.25	0.14±0.01	12.49±4.05
Spring	26.56±0.51	34.49±0.11	1.06±0.24	0.03±0.09	1.13±0.20	0.46±0.12	18.22±3.86
Summer	29.18±1.50	33.65±0.34	1.06±0.16	0.12±0.08	0.66±0.21	0.31±0.06	14.98±2.32
Autumn	27.19±0.17	33.99±0.07	0.98±0.95	0.15±0.13	0.34±0.21	0.18±0.08	13.37±4.11

and roots, as well as sandy substrate, were taken to the laboratory. For mat samples, a 100-cm^2 section was cut from the surrounding mat with a scalpel and a thin sheet of plywood was inserted horizontally beneath the mat at a depth of ~4 cm. The 100 cm^2 section was lifted from the underlying environment and transported to the laboratory for analysis. Enumeration of planktonic and intertidal nitrogen-fixing cyanobacteria was conducted under an Olympus BX-41 fluorescence microscope (Japan). *Trichodesmium* spp. was identified to species level according to its morphology as described by Umezaki (1974) and Carpenter *et al.,* (1993). Nitrogen-fixing cyanobacteria from intertidal samples were identified to genus level, as described by Rippka *et al.,* (1979).

The acetylene reduction method, described by Capone (1993), was used to estimate the N_2 fixation rates. For water column samples, 50 to 100 *T. thiebautii* colonies were gently pipetted from the concentrate and transferred to filtered seawater in 50 ml conical flasks, which were sealed by rubber serum stoppers. There were 7 to 11 replicates for each sample. Samples from intertidal populations were transferred to the laboratory for analysis within 30 min of being collected. In the laboratory, 5 to 10 replicates for each sample were placed in plexiglass cores (100 mm diameter, 1 l volume) and sealed with rubber stoppers. Five percent of the air phase was withdrawn from the cores and replaced with an identical volume of acetylene gas generated from calcium carbide. One core was used as a control to see whether the sediments produced ethylene gas without adding acetylene gas. The conical flasks and plexiglass cores were incubated for 17-20 h in outdoor incubators located around 500 m from Sanya Bay. Neutral screens and circulating water were used to simulate *in situ* conditions of light and temperature. Gas samples was taken once every two hours by a 100-μl gas-tight syringe and analyzed immediately to determine the ethylene concentration with a Chinese-made SQ-204-type gas chromatograph (GC) fitted with a flame ionization detector (FID). A 2 m stainless steel Poropak T-filled column kept at 70°C was used to separate the gases. The carrier gas was high-purity nitrogen. Ethylene production rates were calculated on an areal basis (Brad *et al.,* 1993; Bruns *et al.,* 2002; Fu and Bell, 2003). A conversion factor of 4:1 was assumed when the ethylene production rates were converted to nitrogen fixation rates (Capone, 1993; Montoya *et al.,* 1996; Postgate, 1982). The theoretical acetylene reduction to nitrogen fixation conversion ratio is 3:1 (Capone, 1993). However, as the hydrogenase activity of nitrogenase is blocked by C_2H_4 and produces 1 mol of H_2 per mol of N_2 fixed, a ratio of C_2H_4/N_2=4:1 was assumed to be more appropriate (Postgate, 1982; Montoya *et al.,* 1996). For planktonic cyanobacteria, the nitrogen fixation rate unit was nmol N h^{-1} $colony^{-1}$, and for benthic populations it was μmol N h^{-1} m^{-2}.

The SPSS 13.0. ANOVA test was used to investigate the significance of differences between seasons and nitrogen-fixing populations and communities. A Pearson correlation analysis was performed to relate nitrogen fixation rates to the environmental variables. Probabilities (*P*) of <0.05 were considered to be significant. Surfer 8.0, SigmaPlot, 2001 and Microsoft Word were used to make the figures.

RESULTS

Environmental parameters

Environmental parameters such as water temperature, salinity and inorganic nutrients in Sanya Bay are summarized in Table 1. Temperature and salinity showed seasonal variation. In winter, the average water temperature was 23.49ºC, but it increased to 29.18ºC in summer. Mean water salinity rose as high as 34.41 in winter and was at its lowest in summer, averaging 33.65. These variations are related to the regional climate of Sanya Bay, which has a rainy season (April-October) and a dry season (November-March). From May to August, during the

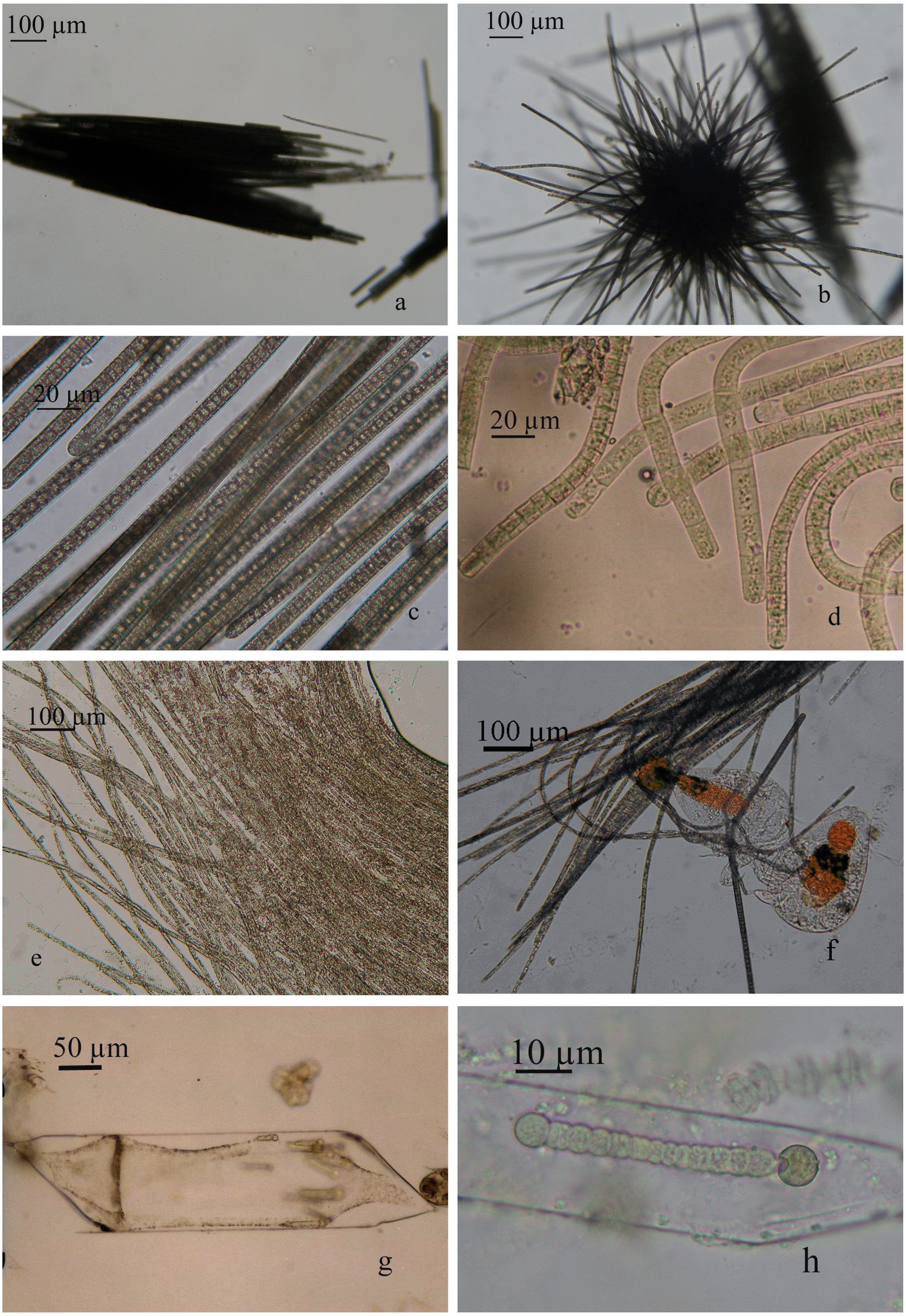

FIG. 2. – Photomicrographs of the main pelagic nitrogen fixing cyanobacteria detected in Sanya Bay, South China Sea. a. *Trichodesmium erythraeum* colony (tuft); b. *Trichodesmium thiebautii* colony (puff); c. *Trichodesmium erythraeum* filaments; d. *Trichodesmium thiebautii* filaments; e. *Trichodesmium* autolysis; f. *Trichodesmium* grazed by zooplanktons; g. *Richelia* (endosymbiont in the diatom *Rhizosolenia*); h. Morphology of *Richelia*.

TABLE 2. – Composition and abundance (10^3 trichomes m^{-2}) of planktonic nitrogen fixing cyanobacteria at fixed station S03 in 2003 (ND means not detected).

	Jan. 2	Feb. 21	Mar. 12	Apr. 10	May. 24	Jun. 10	Jul. 31	Aug. 9	Sept. 24	Oct. 7	Nov. 19	Dec. 11
T .thiebautii	2.4	4.6	5.9	125.0	2060.0	20.3	1.1	8.8	156.0	886.0	1.4	3.8
T. erythraeum	ND	1.0	ND	2.3	ND	ND	ND	ND	ND	2.0	ND	1.1
R. clevei	ND	ND	ND	ND	ND	0.4	0.8	0.6	0.4	ND	ND	ND
R. castracanei	ND	ND	ND	ND	ND	ND	ND	4.0	2.0	1.0	ND	ND
R. styliformis	ND	ND	ND	ND	ND	1.0	ND	1.4	1.6	ND	ND	ND

Southwest Monsoon, there is wind-driven upwelling and the salinity of the Bay is affected by cold water coming from outside the bay, rainfall and freshwater runoff from the Sanya river. These last forcings continue from September to October (Dong *et al.,* 2002a; Huang *et al.,* 2003). From November to March, during the dry season, Sanya Bay salinity is mainly influenced by river runoff. The mean concentrations of dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP) and silicate (SiO_3-Si) were 1.88 μmol l^{-1}, 0.27 μmol l^{-1} and 14.77 μmol l^{-1} respectively. During our investigation, NO_3^--N was the dominant DIN form, followed by NH_4^+-N. Our results and those of other research groups have shown that N is the main limiting nutrient, especially during summer and autumn (Dong *et al.,* 2002a; Huang *et al.,* 2003).

Pelagic cyanobacteria

We found two genera of filamentous pelagic cyanobacteria during the research cruises, *Trichodesmium* and *Richelia* (endosymbiont in the diatom *Rhizosolenia*) (Fig. 2). There were two species of *Trichodesmium, Trichodesmium thiebautii* and *Trichodesmium erythraeum*. As previously reported for the Caribbean and southwest Sargasso Sea (Carpenter and Price, 1977), *T. thiebautii* was the dominant *Trichodesmium* species in Sanya Bay (Table 2). Most *T. thiebautii* occurred as colonial aggregates (tufts), but occasionally spherical colonies (puffs) or free filaments were found (Fig. 2). Cells of *T. thiebautii* averaged 5 μm (4.5-5.38 μm) in length and 10 μm (7.5-13 μm) in width. The average numbers of trichomes per colony and of cells per trichome were 97 and 98 respectively. An in situ experiment in which *Trichodesmium* colonies were incubated within a suspended phytoplankton net (10 L, 20 μm mesh) showed that 95% of *Trichodesmium* in the net disappeared between day 3 and 7, probably due to autolysis or grazing by zooplankton (Fig. 2). The annual average abundance of *T. thiebautii* throughout the monthly sampling at fixed station S03 (Table 2) was 273×10^3 trichomes m^{-2}, with maximum and minimum concentrations of 2060×10^3 and 1.14×10^3 trichomes m^{-2} respectively. High abundance of *Trichodesmium* spp. was detected during the spring (April-June) and autumn (September–October) intermonsoon periods. *Richelia* sp. was found in the cells of three species of *Rhizosolenia*, *R. Clevei, R. castracanei* and *R. styliformis*, but was not abundant (its maximum density was 6.0×10^3 trichomes m^{-2}) (Table 2). About 1-3% of the above mentioned *Rhizosolenia* species had Richelia and there were about 3 to 11 filaments of *Richelia* sp. endosymbiont per *Rhizosolenia* cell.

The results of a two-way ANOVA test indicated significant seasonal and spatial variations of *Trichodesmium* spp. abundance ($P<0.01$) in the four surveys of stations S01 to S11. Abundance of *Trichodesmium* was lowest during the January cruise (Fig. 3). *Trichodesmium* was not detected in any of the Coastal Bay stations, while in the Outer Bay stations its density averaged 5.25×10^4 trichomes m^{-2} and ranged from 3.02 to 10.98×10^4 trichomes m^{-2}. In April, the highest abundance occurred at station S10, with 2040.97×10^4 trichomes m^{-2}. Average *Trichodesmium* densities were 686.37×10^4 trichomes m^{-2}, for Outer Bay stations, 7.97 to 363.98×10^4 trichomes m^{-2} for Mid Bay stations and less than 28.99×10^4 trichomes m^{-2} for Coastal Bay stations. In August, there were no significant differences in *Trichodesmium* abundance between the three sampling areas (Fig. 3). Mean density averaged 27.49 $\times 10^4$ trichomes m^{-2} with a range of 3.40-64.63 $\times 10^4$ trichomes m^{-2}. As in the other three cruises, the peak density of *Trichodesmium* occurred in the Outer Bay stations during October (Fig. 3). The *Trichodesmium* standing crop averaged 26.18×10^4 trichomes m^{-2} in the Outer Bay, while the mean density in the Coastal Bay stations was as low as 8.13×10^4 trichomes m^{-2}. The average nitrogen fixation rate for *T. thiebautii* was 0.27 nmol N h^{-1} colony^{-1} (Table 3), and ranged from 0.22 to 0.83 nmol N h^{-1} colony^{-1} throughout

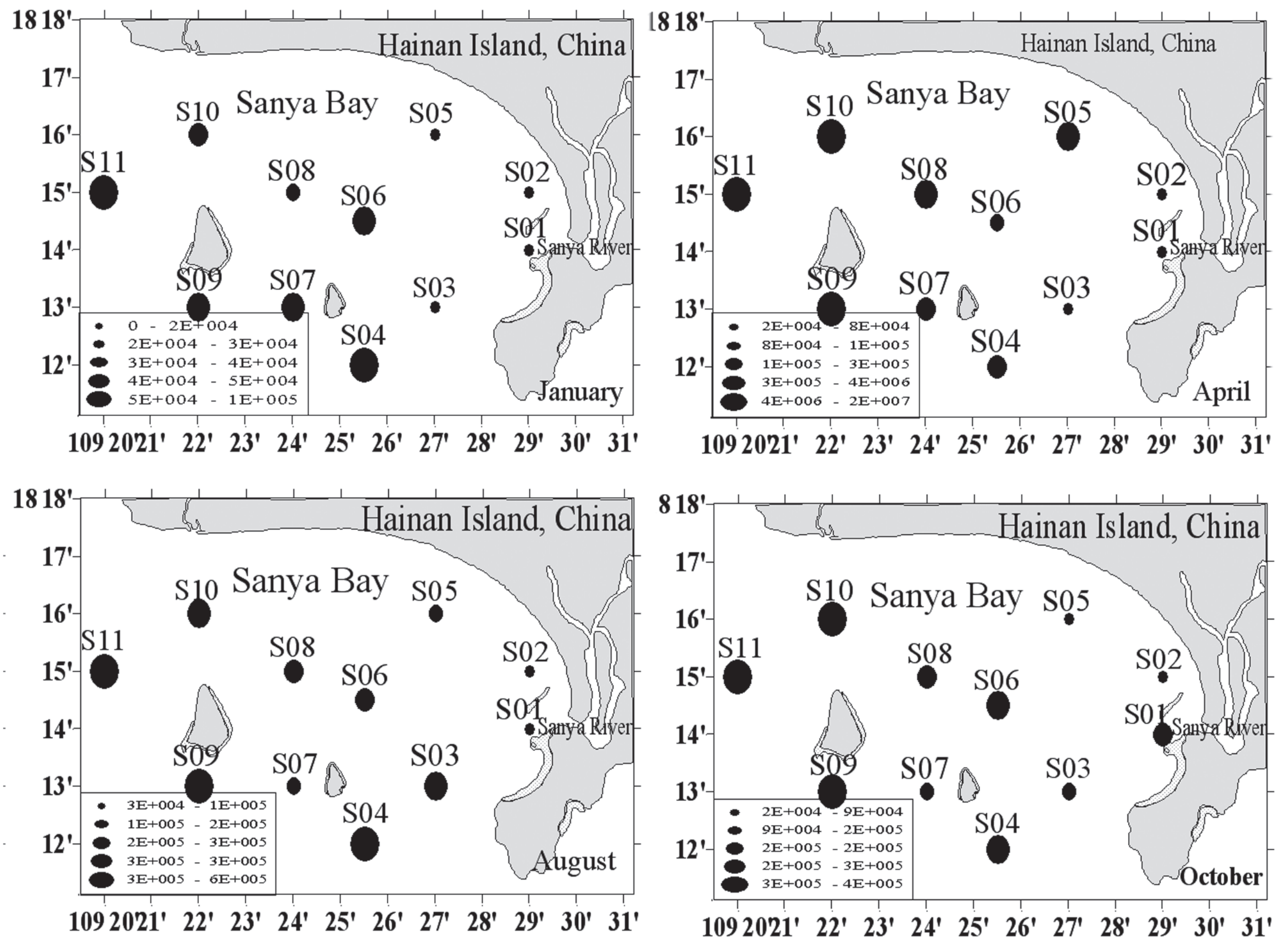

FIG. 3. – Distribution of *Trichodesmium* spp. (trichomes m^{-2}) in Sanya Bay, in the 2004 cruises.

TABLE 3. – Nitrogen fixation rates (nmol N h^{-1} col^{-1})* of *Trichodesmium thiebautii* collected in Sanya Bay.

Date	No. of samples	mean ± Std. deviation	Minimum	Maximum
Nov. 10, 2003	10	0.22±0.05	0.18	0.34
Apr. 9, 2004	8	0.25±0.10	0.19	0.49
Apr. 10, 2004	11	0.22±0.08	0.18	0.45
Jun. 5, 2004	7	0.27±0.12	0.16	0.53
Oct. 11, 2004	9	0.29±0.13	0.20	0.63
Oct. 12, 2004	10	0.34±0.11	0.19	0.53
Sept. 20, 2005	10	0.29±0.20	0.13	0.83
Overall mean		0.27		

* C_2H_4/N_2 ratio = 4:1.

the study period. The maximum rate 0.83 nmol N h^{-1} col^{-1} was recorded in April 2004 in an experiment containing only *T. thiebautii* colonies. However, there was no significant seasonal pattern on a per colony rate basis (t=-0.099; P=0.89>0.05; n=64).

The amount of new nitrogen *Trichodesmium* introduced into Sanya Bay can be estimated from trichome concentrations and nitrogen fixation rates. According to acetylene reduction measurements taken from December 2003 to September 2005, the average pelagic nitrogen fixation rate in Sanya Bay was 0.27 nmol N col^{-1} h^{-1} in samples containing only colonies. If we accept that *Trichodesmium* fixes nitrogen only in sunlight hours (Saino and Hattori, 1982), the daily rate would be 3.24 nmol N col^{-1} day^{-1}. According to *Trichodesmium* densities observed at the sampling stations, estimated nitrogen fixation rates varied between 0 and 0.41×10^{-2} mmol N m^{-2} day^{-1} in Coastal Bay stations (Table 4), between 0.06×10^{-2} and 4.27×10^{-2} mmol N m^{-2} day^{-1} in Mid Bay stations

TABLE 4. – Estimated *Trichodesmium* nitrogen fixation rate and its contribution to primary production in Sanya Bay.

Stations	Cruise	*Trichodesmium* (10^4 trichomes m^{-2})	N fixation rate (10^{-2} mmol N m^{-2} day^{-1})*	Primary production (mmol C m^{-2} day^{-1})	Contribution to primary production (%)
Coastal Bay	Jan. 12	0	0	46.37	0
	Apr. 14	12.33	0.41	204.87	0.01
	Aug. 18	8.26	0.28	192.89	0.01
	Oct. 22	8.13	0.27	69.51	0.03
Mid Bay	Jan. 12	1.88	0.06	33.63	0.01
	Apr. 14	127.98	4.27	63.12	0.45
	Aug. 18	26.59	0.89	181.45	0.03
	Oct. 22	15.40	0.51	56.92	0.06
Outer Bay	Jan. 13	5.25	0.18	38.09	0.03
	Apr. 13	686.37	22.93	92.77	1.63
	Aug. 19	39.57	1.32	87.04	0.10
	Oct. 23	26.18	0.87	70.58	0.08

*C_2H_4/N_2 ratio as in Table 3.

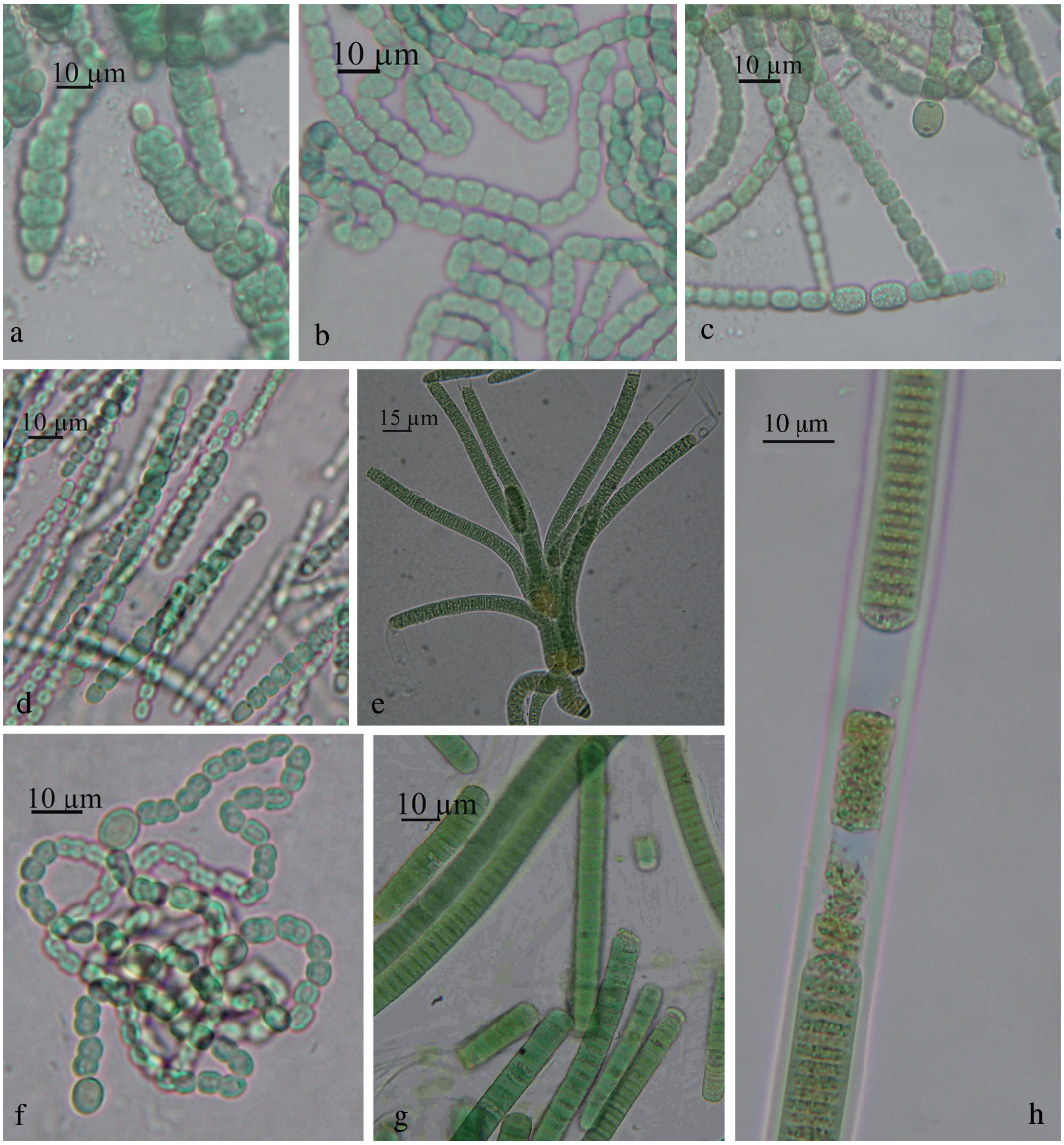

FIG. 4. – Photomicrographs of the main benthic nitrogen-fixing cyanobacteria detected in intertidal communities of Sanya Bay, South China Sea. (a, *Anabaena* sp. strain MCT2; b, *Anabaena* sp. strain MCT4; c, *Anabaena* sp. strain MCT5; d, *Anabaena* sp. strain MCT17; e, *Calothrix* sp. strain MCT1; f, *Nostoc* sp. strain MCT6; g, *Oscillatoria* sp. strain MCT7; h, *Lyngbya* sp. strain MCT6. d, f, isolated from RR communities; c, g, h, isolated from SM communities; a, b, e, isolated from MM communities).

and between 0.18 and 22.93×10^{-2} mmol N m^{-2} day^{-1} in Outer Bay stations (Table 4). Based on an *f*-ratio from 0.14 to 0.47, as reported by Chen *et al.,* (2003) for the South China Sea, *Trichodesmium* may contribute up to 11.64% of the new primary production of the Outer Bay. The fraction of primary production supported by nitrogen fixation in the Coastal Bay stations was much lower (Table 4). However, because *Trichodesmium* blooms were not encountered during our cruises, our estimates only reflect input under non-bloom conditions.

Benthic nitrogen fixing cyanobacteria

The shallow reef systems of Sanya Bay were characterized by calcareous algal communities thriving on rock surfaces and in cracks. Often, cyanobacteria dominated these communities. *Anabaena* sp. strain MCT17 (Fig. 4d) and *Nostoc* sp. strain MCT6 (Fig. 4f) were isolated from the limestone surface and found to be able to fix nitrogen. Six different strains of cyanobacteria were isolated from the microbial mats and two of them are now available as pure cultures. The heterocystous filamentous cyanobacterial genus *Calothrix* sp. strain MCT1 (Fig. 4e), which presented ellipsoidal or spherical heterocysts at the base or within the filaments, was cultured in the marine nitrogen-fixing medium ATCC1077. The filamentous *Anabaena* sp. strain MCT2 (Fig. 4a) was cultured in medium ATCC819.

A two-way ANOVA test was applied to investigate the significance of the variations of nitrogen fixation rates in intertidal communities. Significant differences were detected between seasons (P=0.025<0.05) and between communities (P=0.004<0.01). The highest nitrogen fixation rate was found in the MM (microbial mats) community and the lowest in the RR (reef and rocky sediments) community. All communities presented the highest nitrogen fixation activity in summer and the lowest in winter (Fig. 5). The average nitrogen fixation rate of the MM population was 55.89 μmol N h^{-1} m^{-2} in summer and 13.46 μmol N h^{-1} m^{-2} in winter. The samples from RR sediments showed the lowest nitrogen fixation rates, especially in winter, when no nitrogen fixation was detected in nearly any of the samples. For SM (seagrass and macroalgae ecosystem) communities, the highest nitrogen fixation rate was obtained in summer with a mean of 35.07 μmol N h^{-1} m^{-2}. The new nitrogen contributed by cyanobacteria to the intertidal zone through fixation can be estimated as 0.04-0.39, 0.11-0.67, 0.08-0.50, and 0.01-0.16 m mol N m^{-2} day^{-1} to the coastal system in spring, summer, autumn and winter respectively (Table 5).

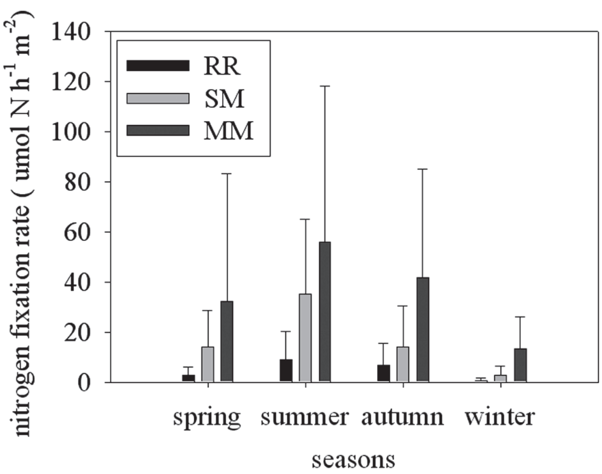

Fig. 5. – Seasonal variations in intertidal nitrogen fixation rates (RR: reefs and rocky sediments, SM: Seagrass and macroalgae population, MM: microbial mats) in Sanya Bay. C_2H_4/N_2 as in Table 3. Error bars denote standard deviation.

Table 5. – Estimation of nitrogen fixation rate (mmol N m^{-2} day^{-1})* of intertidal communities in Sanya Bay. RR= Reefs and rocky sediments; SM= Seagrass and macroalgal communities; MM= Microbial mats.

	Spring	Summer	Autumn	Winter
RR	0.04	0.11	0.08	0.01
SM	0.17	0.42	0.17	0.03
MM	0.39	0.67	0.50	0.16

* C_2H_4/N_2 ratio as in Table 3.

DISCUSSION

The planktonic nitrogen fixing cyanobacteria in Sanya Bay were *T. thiebautii, T. erythraeum* and *Richelia* sp. *T. thiebautii,* usually occurred as colonial aggregates and was the most abundant species during our cruises, in agreement with previous observations from the North Atlantic Ocean and Japan Sea (Carpenter and Price, 1977). Here, we report on the standing crops and nitrogen fixation rates of *Trichodesmium* spp. Densities were consistent with previous observations of 10^4 trichomes m^{-2} (Chen, 2003), but were low compared to reports from the Great Barrier Reef Lagoon (Bell *et al.,* 1999) and the tropical N Atlantic (Carpenter and Romans, 1991). The abundance of *Trichodesmium* spp. showed large spatial and seasonal variations; it was lowest in the

TABLE 6. – Pearson correlation coefficients between nitrogen fixations rates and environmental variables. RR= Reefs and rocky sediments; SM= Seagrass and macroalgal communities; MM= Microbial mats. *P<0.05.

Variables	Temperature	Salinity	NO_3-N	NO_2-N	NH_4-N	PO_4-P	SiO_3-Si	N/P
RR	0.943*	-0.938*	0.129	0.777	-0.674	0.112	0.044	-0.601
SM	0.936*	-0.842	0.551	0.438	-0.285	0.389	0.295	-0.685
MM	0.993*	-0.860	0.306	0.629	-0.515	0.327	0.261	-0.755
Trichodesmium	0.958*	-0.845	-0.418	0.872	-0.880	-0.133	-0.126	-0.423

TABLE 7. – Comparison of nitrogen fixation rate estimates in various intertidal communities.

Types	Locality	N-fixation rate (mg N m^{-2} d^{-1})	Reference
Coral reef communities	Sanya Bay (RR)	0.14-1.51	This study
	One Tree Island	2.19-4.38	Larkum *et al.,* (1988)
	Eilat	93.16	Shashar *et al.,* (1994)
	Lizard Island	1.86-8.38	Burris (1976)
	Tikehau	1.4-8	Charpy-Rouhaud *et al.,* (1997)
	Great Barrier Reef	0.77-4.99	Wilkinson *et al.,*(1984)
Seagrass ecosystems	Sanya Bay (SM)	0.48-5.89	This study
	Vaucluse Shores	3.9-6.5	Capone (1982)
	Great South Bay	5.2	Capone (1982)
	Bassin d'Arcachon	1.20-3.01	Welsh *et al.,* (1996)
	Florida	0.03	McRoy *et al.,* (1973)
	Biscayne Bay	5-24	Capone and Taylor (1980)
	Bimini Harbour, Bahamas	5.1-9.0	Capone *et al.,* 1979
	Barbados	27.40-136.99	Patriquin and Knowles (1972)
	Moreton Bay, Australia	10-40	O'Donobue *et al.,* (1991)
Cyanobacteria mats	Sanya Bay (MM)	2.26-9.39	This study
	Texas Gulf coast	10.96	Gotto *et al.,* (1981)
	Tikehau	8	Charpy-Rouhaud *et al.,* (1997)
	Tomales Bay	6-79	Joye and Paerl (1994)
	Mellum Island	2.19-4.11	Stal *et al.,* (1984)
	Yucatan Peninsula	1.19-6.25	Eliska and Jaroslava (2000)

January cruise and highest in the April cruise. The variations in the *Trichodesmium* spp. standing crop between the Sanya Bay regions ranked as follows: Outer Bay>Mid Bay>Coastal Bay (Table 4 and Fig. 3). Seawater temperature has been recognized as a major factor controlling *Trichodesmium* abundance (Chen *et al.,* 2003; Lugomela *et al.,* 2002). In January, the scarcity of *Trichodesmium* in Sanya Bay, especially in the coastal stations, was most probably a result of the low temperatures. As the temperature increased in April, *Trichodesmium* became more abundant (Table 4). However, no relationship with seawater temperature could be established for the Outer Bay samples. The results of the monthly sampling at station S03 in 2003 showed that, generally, *Trichodesmium* abundance increased greatly during the period from April to May, which coincided with the transition from the NE to the SW monsoon, reaching another peak with the transition from the SW to the NE monsoon (Sep–Oct). This observation suggests that monsoons may play a significant role in the distribution of *Trichodesmium* in Sanya Bay.

The nitrogen fixation rates obtained for *T. thiebautii* in Sanya Bay (0.22 to 0.83 nmol N h^{-1} col^{-1}, with an average of 0.27 nmol N h^{-1} $colony^{-1}$) were similar to those reported by Carpenter *et al.,* (1993). The availability of combined nitrogen and other essential nutrients (e.g., phosphorus and iron), temperature, and the quality and quantity of light can all affect *Trichodesmium* nitrogen fixation rates in nature (Eisele *et al.*, 1989; Capone *et al.*, 1997; Letelier and Karl, 1998; Bissett *et al.*, 1999; Sañudo-Wilhelmy *et al*., 2001; Mills *et al.,* 2004; Mulholland and Bernhardt, 2005). Our results showed that the nitrogen fixation rate of *Trichodesmium* was positively correlated to temperature (Table 6); however, there was no obvious seasonal variation of colony-specific nitrogen fixation rates, in agreement with the results from Bell *et al.,* (1999). No significant correlation was detected between *Trichodesmium* nitrogen fixation rates and nutrient concentrations in the Sanya Bay water column (Table 6). *Trichodesmium* use N_2 as their primary N source and therefore it has been suggested that dissolved inorganic P (DIP) may limit the growth of *Trichodesmium*.

However, *Trichodesmium* is capable of hydrolyzing dissolved organic P (DOP) compounds and the inorganic products from hydrolysis may provide an additional source of P for growth. Mulholland *et al*., (2002) showed that if the supply of appropriate DOP substrates is adequate, DOP may represent an important P source for *Trichodesmium* growth. Another factor not considered in our assay, which could also affect the abundance and nitrogen fixation activity of *Trichodesmium* in Sanya Bay, is the intensity of aeolian dust deposition (Sarglo *et al.,* 2001; Mulholland *et al.,* 2002; Mulholland and Bernhardt *et al.,* 2005).

In addition to its incremental contribution to algal biomass and primary production, *Trichodesmium* may also enhance overall primary and export production by introducing new nitrogen into the euphotic zone (Carpenter *et al.,* 2004). The results obtained by Letelier *et al.,* (1996) and Letelier and Karl (1996) indicated that nitrogen fixation by *Trichodesmium* accounted for 4% of the total primary production of C at the station ALOHA north of Hawaii between 1989 and 1992. In the Outer Bay region, nitrogen fixation by *Trichodesmium* contributed from 0.03 to 1.63% of the total primary production, a lower percentage than that obtained by Carpenter *et al.,* (2004) in the tropical North Atlantic Ocean, but consistent with the results obtained by Chang *et al*., (2000), who estimated that *Trichodesmium* nitrogen fixation accounted for 0.2% to 2.3% of total primary production in Kuroshio waters, near Taiwan. With *f* ratios ranging from 0.14 to 0.47 (Chen *et al.,* 2003), our N_2 fixation estimates for the Outer Bay could sustain up to 11.64% of the new primary production.

The dominant benthic cyanobacteria in the intertidal communities of Sanya Bay included non-heterocystous cyanobacteria like *Oscillatoria* spp. and *Lyngbya* spp., and heterocystous ones such as *Calothrix* spp., *Anabaena* spp. and *Entophysalis* sp. Our study detected obvious seasonal variations, especially among the dominant species, which could play a significant role in nitrogen fixation. Most cyanobacteria were very abundant in summer and disappeared in winter (Dong *et al.,* 2006). This was the main reason for the obvious seasonal variations in nitrogen fixation rates for the RR, SM and MM communities. The statistical results of this study showed a positive correlation between nitrogen fixation rates and temperature for the three intertidal community groups (P<0.05) (Table 6). This was consistent with previous results for *Calothrix* sp. and *Lyngbya* sp., which were the dominant nitrogen fixing cyanobacteria in our MM and SM communities respectively. Both species, isolated from natural populations, had higher nitrogen fixation rates at 30ºC than at 20ºC (Zhang *et al.,* 2006). The nitrogen fixation rate of the three intertidal community types showed negative correlation coefficients with salinity, but the correlation was only significant for the RR community (P<0.05). There were no significant relationships between benthic nitrogen fixation rates and the nutrient concentrations in the water column (Table 6.). Laboratory studies show that increased N availability often suppresses the nitrogen fixation rate, while increased P availability frequently stimulates the nitrogen fixation rate (Madigan *et al.,* 2003). When total dissolved N reached above 10 μM, nitrogen fixation was not detectable (Doyle and Fisher, 1994). However, during our investigation, the concentration of dissolved inorganic nitrogen ranged from 1.47 to 2.22 μmol l^{-1} and N was the limiting nutrient in Sanya Bay.

Nitrogen fixation by benthic cyanobacteria can be a significant source of biologically available nitrogen for other components of the ecosystem (Gotto *et al.,* 1981; Eliska and Jaroslava, 2000). Available estimates of nitrogen fixation rates in various intertidal communities cover a wide range (Table 7) with values up to 93.16 mg N m^{-2} d^{-1} in coral reef communities in Eilat (Shashar *et al.,* 1994) and 27.40-136.99 mg N m^{-2} d^{-1} in seagrass ecosystems in Barbados (Patriquin and Knowles, 1972). Our values for Sanya Bay fall in the lower part of the range for reef and seagrass communities and in the upper part of the range for cyanobacterial mats (Table 7). In summer the contribution of nitrogen fixation estimated for SM and MM populations was as high as 0.42 and 0.67 mmol N m^{-2} d^{-1} respectively, which represents a significant contribution to the coastal ecosystems in Sanya Bay. On a surface-specific basis, the nitrogen fixation rates of benthic cyanobacteria were much higher than those of planktonic cyanobacteria. However, the open water area in the bay was much larger than that occupied by benthic cyanobacteria. Therefore, we can conclude that nitrogen fixation by planktonic cyanobacteria plays an important role in the Mid Bay and Outer Bay stations while nitrogen fixation by benthic cyanobacteria represents a significant contribution in the intertidal areas of the bay where these cyanobacteria exist.

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